

Supporting Information

Eutectic solvent platform for the selective isolation and one-year stabilisation of astaxanthin from crustacean shell waste

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Table S1. Coded and decoded matrices for the 2^2 factorial planning carried out through a Response Surface Methodology considering firstly astaxanthin concentration as the response variable and later considering total proteins concentration as the response variable.

Run	Coded Matrix		Decoded matrix	
	X ₁	X ₂	Time (min)	SLR
1	-1	-1	30	0.100
2	1	-1	70	0.100
3	-1	1	30	0.300
4	1	1	70	0.300
5	-1.41	0	22	0.200
6	1.41	0	78	0.200
7	0	-1.41	50	0.059
8	0	1.41	50	0.341
9	0	0	50	0.200
10	0	0	50	0.200
11	0	0	50	0.200

Table S2. Composition and component properties of the systems investigated for DES preparation. All systems were prepared at an HBA:HBD molar ratio of 1:2 at 80 °C.

HBA	HBD	HBA molar mass (g mol ⁻¹)	HBD molar mass (g mol ⁻¹)	HBA CAS No.	HBD CAS No.	HBA water content (%)	HBD water content (%)	DES formation	Liquid at 25 °C
[Ch]Cl	Acetic acid	139.62	60.05	67-48-1	64-19-7	0.43	0.06	✓	✓
[N _{4,4,4,4}]Br		322.37		1643-19-2		0.18		✓	✓
[Ch]Cl	Lactic acid	139.62	90.08	67-48-1	50-21-5	0.43	15.5	✓	✓†
[N _{4,4,4,4}]Br		322.37		1643-19-2		0.18		✓	✓†
[Ch]Cl	Malonic acid	139.62	104.06	67-48-1	141-82-2	0.43	0.39	✓	✓
[N _{4,4,4,4}]Br		322.37		1643-19-2		0.18		✓	✓
[Ch]Cl	Octanoic acid	139.62	144.21	67-48-1	124-07-2	0.43	0.13	✗	—
[N _{4,4,4,4}]Br		322.37		1643-19-2		0.18		✓	✓
[Ch]Cl	Lauric acid	139.62	200.32	67-48-1	143-07-7	0.43	0.48	✗	—
[N _{4,4,4,4}]Br		322.37		1643-19-2		0.18		✓	✗
[Ch]Cl	Ethylene glycol	139.62	62.07	67-48-1	107-21-1	0.43	0.04	✓	✓
[N _{4,4,4,4}]Br		322.37		1643-19-2		0.18		✓	✓
[Ch]Cl	Glycerol	139.62	92.09	67-48-1	56-81-5	0.43	0.13	✓	✓
[N _{4,4,4,4}]Br		322.37		1643-19-2		0.18		✓	✗
[Ch]Cl	Butanol	139.62	74.12	67-48-1	71-36-3	0.43	0.17	✗	—
[N _{4,4,4,4}]Br		322.37		1643-19-2		0.18		✓	✓
[Ch]Cl	Hexanol	139.62	102.17	67-48-1	111-27-3	0.43	0.08	✗	—
[N _{4,4,4,4}]Br		322.37		1643-19-2		0.18		✓	✓

Notes: HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; [Ch]Cl, choline chloride; [N_{4,4,4,4}]Br, tetrabutylammonium bromide. ✓, yes; ✗, no.

† Liquid at 25 °C before extraction, but became solid during extraction.

Table S3. Experimental and fitted data of astaxanthin concentration (response variable) and respective residues with two-independent variables (time and solid-liquid ratio) for [N_{4,4,4,4}]Br:butanol (1:2 molar ratio).

Run	Time (min)	SLR	Castaxanthin (µg mL ⁻¹)	Castaxanthin (µg mL ⁻¹)	Residues
			Experimental values	Fitted values	
1	30	0.1	13.09	10.83	2.26
2	70	0.1	11.00	10.77	0.22
3	30	0.3	25.26	23.12	2.14
4	70	0.3	20.09	19.99	0.10
5	22	0.2	18.03	20.67	-2.63
6	78	0.2	18.68	18.42	0.26
7	50	0.059	3.96	5.24	-1.27
8	50	0.341	19.30	20.40	-1.10
9	50	0.2	14.58	16.73	-2.16
10	50	0.2	18.34	16.73	1.60
11	50	0.2	17.32	16.73	0.59

Note: The coefficient of determination was calculated as $R^2 = 1 - \frac{\sum_{i=1}^{11} (y_i - \hat{y}_i)^2}{\sum_{i=1}^{11} (y_i - \bar{y})^2}$, where y_i is the experimental concentration, $\hat{y}_i$ is the fitted concentration, and $\bar{y}$ is the mean experimental concentration across the 11 runs. $R^2 = 0.913$.

Table S4. Experimental and fitted data of total protein concentration (response variable) and respective residues with two-independent variables (time and solid-liquid ratio) for [N_{4,4,4,4}]Br:butanol (1:2 molar ratio).

Run	Time (min)	SLR	Ctotal proteins (mg mL ⁻¹)	Ctotal proteins (mg mL ⁻¹)	Residues
			Experimental values	Fitted values	
1	30	0.1	3.66	3.18	0.48
2	70	0.1	2.72	2.75	-0.04
3	30	0.3	9.93	9.69	0.25
4	70	0.3	9.12	9.40	-0.27
5	22	0.2	7.05	7.53	-0.48
6	78	0.2	7.29	7.03	0.26
7	50	0.059	0.32	0.59	-0.27
8	50	0.341	9.92	9.86	0.06
9	50	0.2	5.67	6.19	-0.52
10	50	0.2	6.32	6.19	0.13
11	50	0.2	6.58	6.19	0.39

Note: The coefficient of determination was calculated as $R^2 = 1 - \frac{\sum_{i=1}^{11} (y_i - \hat{y}_i)^2}{\sum_{i=1}^{11} (y_i - \bar{y})^2}$, where y_i is the experimental concentration, $\hat{y}_i$ is the fitted concentration, and $\bar{y}$ is the mean experimental concentration across the 11 runs. $R^2 = 0.987$.

Table S5. Experimental and fitted data of astaxanthin concentration (response variable) and respective residues with two-independent variables (time and solid-liquid ratio) for [N_{4,4,4,4}]Br:hexanol (1:2 molar ratio).

Run	Time (min)	SLR	Castaxanthin (µg mL ⁻¹)	Castaxanthin (µg mL ⁻¹)	Residues
			Experimental values	Fitted values	
1	30	0.1	10.66	9.64	1.02
2	70	0.1	7.23	9.19	-1.96
3	30	0.3	36.70	34.04	2.65
4	70	0.3	33.39	33.72	-0.32
5	22	0.2	19.89	22.35	-2.46
6	78	0.2	23.56	21.80	1.76
7	50	0.059	4.78	3.97	0.81
8	50	0.341	36.95	38.46	-1.51
9	50	0.2	21.23	21.07	0.16
10	50	0.2	18.93	21.07	-2.15
11	50	0.2	23.08	21.07	2.00

Note: The coefficient of determination was calculated as $R^2 = 1 - \frac{\sum_{i=1}^{11}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{11}(y_i - \bar{y})^2}$, where y_i is the experimental concentration, $\hat{y}_i$ is the fitted concentration, and $\bar{y}$ is the mean experimental concentration across the 11 runs. $R^2 = 0.973$.

Table S6. Experimental and fitted data of total protein concentration (response variable) and respective residues with two-independent variables (time and solid-liquid ratio) for [N_{4,4,4,4}]Br:hexanol (1:2 molar ratio).

Run	Time (min)	SLR	C _{total proteins} (mg mL ⁻¹)	C _{total proteins} (mg mL ⁻¹)	Residues
			Experimental values	Fitted values	
1	30	0.1	0.81	1.10	-0.29
2	70	0.1	0.68	0.94	-0.25
3	30	0.3	3.49	3.73	-0.24
4	70	0.3	3.55	3.75	-0.21
5	22	0.2	2.65	2.38	0.27
6	78	0.2	2.51	2.28	0.23
7	50	0.059	0.79	0.51	0.28
8	50	0.341	4.57	4.35	0.22
9	50	0.2	2.25	2.21	0.04
10	50	0.2	1.87	2.21	-0.35
11	50	0.2	2.52	2.21	0.30

Note: The coefficient of determination was calculated as $R^2 = 1 - \frac{\sum_{i=1}^{11}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{11}(y_i - \bar{y})^2}$, where y_i is the experimental concentration, $\hat{y}_i$ is the fitted concentration, and $\bar{y}$ is the mean experimental concentration across the 11 runs. $R^2 = 0.954$.

Table S7. Experimental and fitted data of astaxanthin concentration (response variable) and respective residues with two-independent variables (time and solid-liquid ratio) for [N_{4,4,4,4}]Br:octanoic acid (1:2 molar ratio).

Run	Time (min)	SLR	Castaxanthin (µg mL ⁻¹)	Castaxanthin (µg mL ⁻¹)	Residues
			Experimental values	Fitted values	
1	30	0.1	8.60	9.96	-1.36
2	70	0.1	9.01	7.35	1.67
3	30	0.3	27.10	27.13	-0.03
4	70	0.3	34.89	31.89	3.00
5	22	0.2	20.59	19.27	1.32
6	78	0.2	17.81	20.78	-2.97
7	50	0.059	3.56	3.44	0.12
8	50	0.341	31.07	32.84	-1.77
9	50	0.2	17.98	18.84	-0.85
10	50	0.2	16.37	18.84	-2.47
11	50	0.2	22.19	18.84	3.35

Note: The coefficient of determination was calculated as $R^2 = 1 - \frac{\sum_{i=1}^{11}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{11}(y_i - \bar{y})^2}$, where y_i is the experimental concentration, $\hat{y}_i$ is the fitted concentration, and $\bar{y}$ is the mean experimental concentration across the 11 runs. $R^2 = 0.951$.

Table S8. Experimental and fitted data of total protein concentration (response variable) and respective residues with two-independent variables (time and solid-liquid ratio) for [N_{4,4,4,4}]Br:octanoic acid (1:2 molar ratio).

Run	Time (min)	SLR	C _{total proteins} (mg mL ⁻¹)	C _{total proteins} (mg mL ⁻¹)	Residues
			Experimental values	Fitted values	
1	30	0.1	1.49	1.17	0.33
2	70	0.1	0.98	0.51	0.46
3	30	0.3	2.27	1.95	0.32
4	70	0.3	3.23	2.78	0.45
5	22	0.2	1.26	1.55	-0.29
6	78	0.2	1.18	1.67	-0.49
7	50	0.059	0.11	0.51	-0.40
8	50	0.341	2.28	2.66	-0.38
9	50	0.2	1.14	1.02	0.12
10	50	0.2	1.10	1.02	0.08
11	50	0.2	0.83	1.02	-0.19

Note: The coefficient of determination was calculated as $R^2 = 1 - \frac{\sum_{i=1}^{11}(y_i - \hat{y}_i)^2}{\sum_{i=1}^{11}(y_i - \bar{y})^2}$, where y_i is the experimental concentration, $\hat{y}_i$ is the fitted concentration, and $\bar{y}$ is the mean experimental concentration across the 11 runs. $R^2 = 0.819$.

Table S9. Regression coefficients and standard deviation of the 2² factorial planning developed for astaxanthin concentration as the response variable for [N_{4,4,4,4}]Br:butanol (1:2 molar ratio).

	Regression Coefficient	Std. deviation	t-student (5)	p-value
Mean/Interc.	5.081	9.575	0.531	0.618
Time (min)	-0.316	0.276	-1.148	0.303
Time ² (min)	0.004	0.002	1.435	0.211
SLR	151.753	49.692	3.054	0.028
SLR ²	-196.896	98.491	-1.999	0.102
Time (min) x SLR	-0.384	0.583	-0.660	0.539

Table S10. Regression coefficients and standard deviation of the 2² factorial planning developed for total protein concentration as the response variable for [N_{4,4,4,4}]Br:butanol (1:2 molar ratio).

	Regression Coefficient	Std. deviation	t-student (5)	p-value
Mean/Interc.	1.718	1.999	0.859	0.429
Time (min)	-0.149	0.058	-2.595	0.049
Time ² (min)	0.001	0.001	2.665	0.045
SLR	51.365	10.374	4.951	0.004
SLR ²	-48.308	20.562	-2.349	0.066
Time (min) x SLR	0.017	0.122	0.137	0.896

Table S11. Regression coefficients and standard deviation of the 2² factorial planning developed for astaxanthin concentration as the response variable for [N_{4,4,4,4}]Br:hexanol (1:2 molar ratio).

	Regression Coefficient	Std. deviation	t-student (5)	p-value
Mean/Interc.	0.678	10.511	0.065	0.951
Time (min)	-0.139	0.302	-0.458	0.666
Time ² (min)	0.001	0.003	0.465	0.661
SLR	118.727	54.551	2.176	0.081
SLR ²	7.072	108.122	0.065	0.950
Time (min) x SLR	0.015	0.640	0.024	0.982

Table S12. Regression coefficients and standard deviation of the 2² factorial planning developed for total protein concentration as the response variable for [N_{4,4,4,4}]Br:hexanol (1:2 molar ratio).

	Regression Coefficient	Std. deviation	t-student (5)	p-value
Mean/Interc.	0.600	1.551	0.387	0.715
Time (min)	-0.021	0.045	-0.470	0.658
Time ² (min)	0.000	0.000	0.366	0.729
SLR	8.171	8.051	1.015	0.357
SLR ²	10.774	15.957	0.675	0.530
Time (min) x SLR	0.023	0.094	0.244	0.817

Table S13. Regression coefficients and standard deviation of the 2² factorial planning developed for astaxanthin concentration as the response variable for [N_{4,4,4,4}]Br:octanoic acid (1:2 molar ratio).

	Regression Coefficient	Std. deviation	t-student (5)	p-value
Mean/Interc.	8.176	12.377	0.661	0.538
Time (min)	-0.307	0.356	-0.861	0.429
Time ² (min)	0.001	0.003	0.468	0.659
SLR	72.324	64.233	1.126	0.311
SLR ²	-35.311	127.312	-0.277	0.793
Time (min) x SLR	0.921	0.753	1.223	0.276

Table S14. Regression coefficients and standard deviation of the 2² factorial planning developed for total protein concentration as the response variable for [N_{4,4,4,4}]Br:octanoic acid (1:2 molar ratio).

	Regression Coefficient	Std. deviation	t-student (5)	p-value
Mean/Interc.	4.228	2.108	2.005	0.101
Time (min)	-0.109	0.061	-1.795	0.133
Time ² (min)	0.001	0.001	1.368	0.230
SLR	-13.006	10.942	-1.189	0.288
SLR ²	28.494	21.688	1.314	0.246
Time (min) x SLR	0.185	0.128	1.438	0.210

Table S15. Extractive performance of [N_{4,4,4,4}]Br:hexanol (1:2) for astaxanthin, total proteins and minerals, under the optimal conditions (SLR = 0.4, and extraction time = 10 min). Minerals were quantified using incineration of the biomass at 600 °C for 6 h.

Astaxanthin		Total proteins		Minerals (mg g _{shells} ⁻¹)
C _{astaxanthin} (μg mL ⁻¹)	C _{astaxanthin} (μg g _{shells} ⁻¹)	C _{total proteins} (mg mL ⁻¹)	C _{total proteins} (mg g _{shells} ⁻¹)	
27 ± 1	68 ± 2	5.0 ± 0.2	12.5 ± 0.5	0

Table S16. Changes in astaxanthin concentration ($\mu\text{g}_{\text{astaxanthin}} \text{g}_{\text{shells}}^{-1}$) during 365-day storage under different temperature and light exposure conditions.

Conditions	Total astaxanthin content in biomass ($\mu\text{g}_{\text{astaxanthin}} \text{g}_{\text{shells}}^{-1}$)	Day 1	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180	Day 210	Day 240	Day 270	Day 300	Day 330	Day 365
		$\mu\text{g}_{\text{astaxanthin}} \text{g}_{\text{shells}}^{-1}$												
25 °C dark	71.5	61.6	43.4 ± 0.5	43.5 ± 0.8	39.7 ± 0.5	36.0 ± 0.1	35.3 ± 0.6	34.2 ± 0.4	34.1 ± 0.7	31.6 ± 0.4	31.1 ± 0.2	25.9 ± 0.3	28.40 ± 0.08	26.9 ± 0.4
25 °C light			41.6 ± 0.6	38.0 ± 0.3	32 ± 1	27.2 ± 0.6	28.0 ± 0.6	26.0 ± 0.7	25.0 ± 0.7	21.6 ± 0.8	20.5 ± 0.7	16 ± 1	13 ± 2	12 ± 2
40 °C dark			41.3 ± 0.8	37.7 ± 0.6	31 ± 1	25.3 ± 0.6	24 ± 1	22 ± 2	19 ± 2	16 ± 3	16 ± 2	12 ± 2	12 ± 3	12 ± 3
60 °C dark			29 ± 1	13 ± 3	6 ± 2	4 ± 1	4 ± 1					-----		
Control 60 °C dark		47.2						-----						

Note: At lower temperatures the acetic acid extract (control) formed a gel; therefore, stability analysis was performed only at 60 °C. In the control sample, disappearance of the characteristic astaxanthin UV–Vis absorption band occurred after 15 days, indicating complete degradation. In contrast, the DES sample at 60 °C retained the absorption band until 150 days, after which complete degradation was observed and analysis was discontinued.

Table S17. Changes in total proteins concentration ($\text{mg}_{\text{total proteins}} \text{g}_{\text{shells}}^{-1}$) during 365-day storage under different temperature and light exposure conditions.

Conditions	Total proteins content in biomass ($\text{mg}_{\text{total proteins}} \text{g}_{\text{shells}}^{-1}$)	Day 1	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180	Day 210	Day 240	Day 270	Day 300	Day 330	Day 365
		$\text{mg}_{\text{total proteins}} \text{g}_{\text{shells}}^{-1}$												
25 °C dark	68.0	25.6	26.1 ± 0.7	27.5 ± 0.6	27.2 ± 0.2	27 ± 1	27.2 ± 0.7	26.8 ± 0.8	27.1 ± 0.7	27 ± 2	25.2 ± 0.6	27.4 ± 0.9	28.0 ± 0.4	24.5 ± 0.5
25 °C light			25 ± 1	25.7 ± 0.5	26.1 ± 0.5	26 ± 1	27 ± 2	26 ± 0.12	25.1 ± 0.2	24.7 ± 0.9	25 ± 1	25.9 ± 0.2	26.0 ± 0.5	25.4 ± 0.5
40 °C dark			30.0 ± 0.8	32 ± 2	34 ± 5	36 ± 4	36 ± 2	34 ± 1	38.3 ± 0.7	32.7 ± 5	33 ± 5	35 ± 3	37 ± 3	34 ± 3
60 °C dark			30 ± 1	36 ± 3	42 ± 1	43 ± 4	44 ± 7				----			
Control 60 °C dark		52.0	120 ± 4	165 ± 18	247 ± 79.4*					----				

Note: At lower temperatures, the acetic acid extract (control) formed a gel; therefore, stability analysis was conducted only at 60 °C. In the *DES* sample, UV–Vis spectra showed disappearance of the characteristic astaxanthin absorption band after 150 days, indicating complete degradation and termination of further analysis. The control sample developed turbidity after 90 days and was likewise excluded from subsequent analysis.

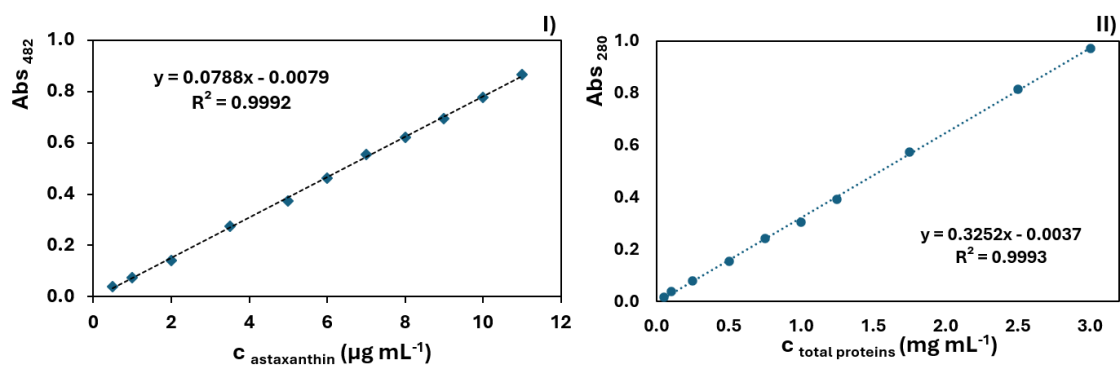


Figure S1. Calibration curves of astaxanthin (I) and total proteins (II).

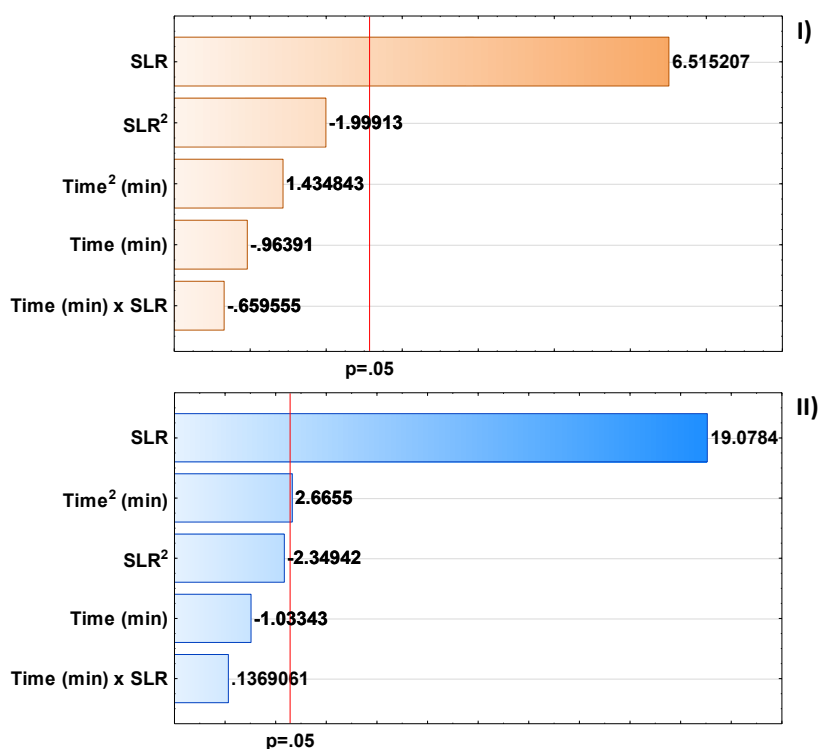


Figure S2. Pareto charts with the influence of SLR and time upon the concentration of astaxanthin (I) and total proteins (II) after the solid-liquid extraction with $[\text{N}_{4,4,4,4}]\text{Br}:\text{Butanol}$ (1:2).

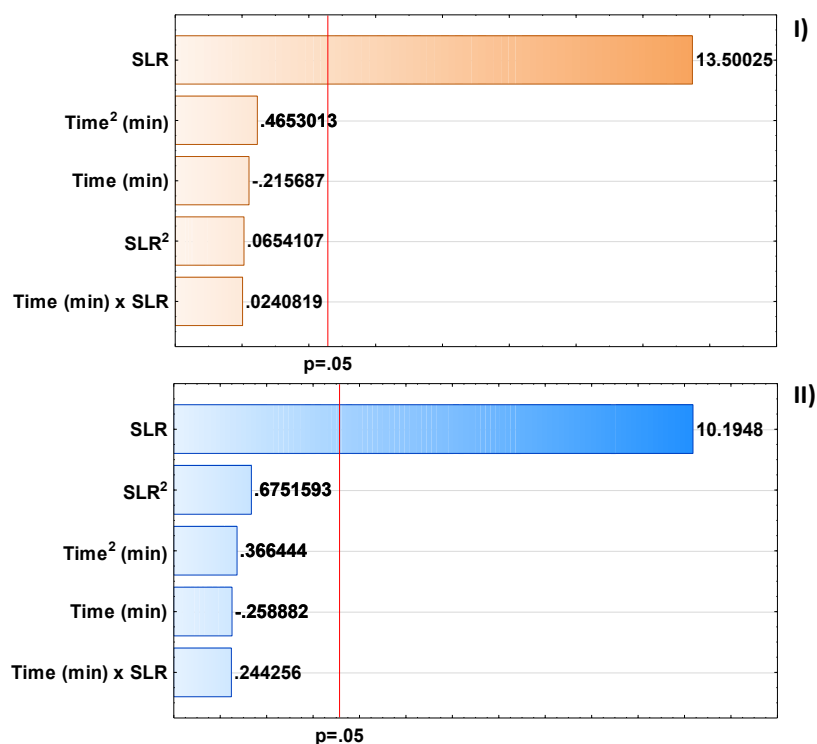


Figure S3. Pareto charts with the influence of SLR and time upon the concentration of astaxanthin (I) and total proteins (II) after the solid-liquid extraction with $[N_{4,4,4,4}]\text{Br}:\text{Hexanol}$ (1:2).

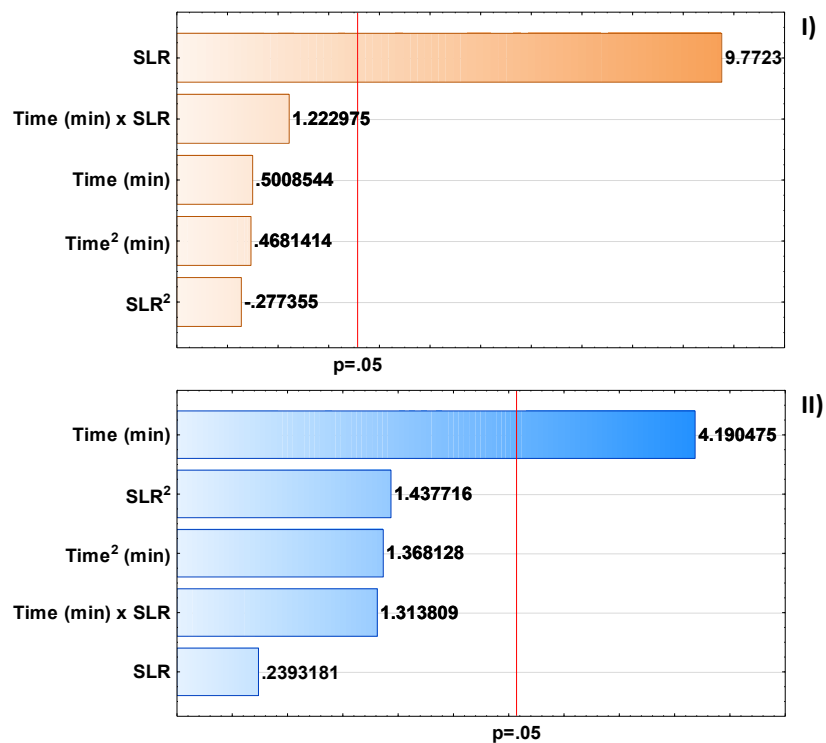


Figure S4. Pareto charts with the influence of SLR and time upon the concentration of astaxanthin (I) and total proteins (II) after the solid-liquid extraction with $[N_{4,4,4,4}]\text{Br}:\text{Octanoic acid}$ (1:2).



Figure S5. Beakers after biomass incineration following a single extraction cycle with $[N_{4,4,4,4}]\text{Br}$:hexanol (1:2). The starting biomass mass was approximately 2.81 g, and no residue was detected after incineration at 600 °C for 6 h, indicating that mineral extraction did not occur.

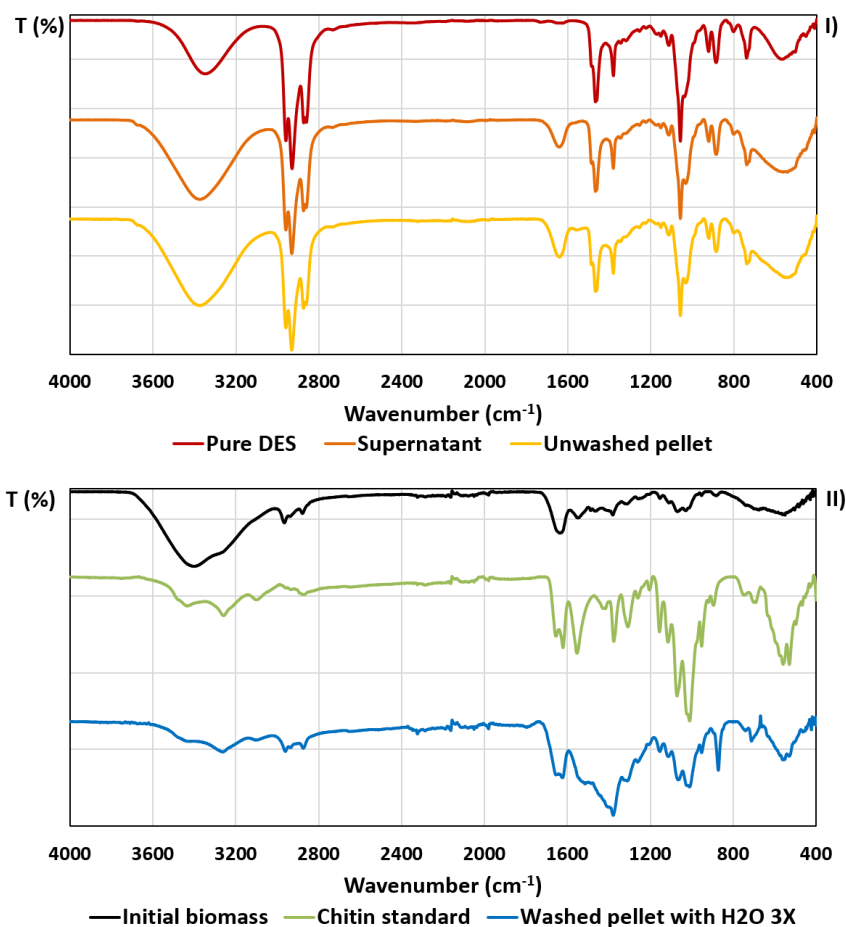


Figure S6. FTIR spectra of the DES extraction fractions used to assess chitin retention after astaxanthin extraction from shrimp shell waste. (I) Pure DES, extraction supernatant, and unwashed pellet. (II) Initial biomass, commercial chitin standard, and pellet after water washing and drying. The characteristic α -chitin bands observed in the washed pellet confirm that chitin remained in the solid fraction during extraction.

FTIR analysis was performed to evaluate whether chitin was co-extracted during astaxanthin recovery. As shown in **Figure S6.I**, the FTIR spectrum of the supernatant is dominated by the characteristic absorption bands of the DES. Nevertheless, a band around 1650 cm^{-1} can be observed, corresponding to the amide I vibration (C=O stretching) of co-extracted proteins. In contrast to proteins, α -chitin typically exhibits a split amide I region, with two characteristic

bands at approximately 1655 cm^{-1} and 1620 cm^{-1} arising from carbonyl groups involved in different hydrogen-bonding environments within its crystalline structure. The absence of this characteristic doublet in the supernatant indicates that no detectable amount of chitin was extracted under the conditions employed. The FTIR spectrum of the unwashed pellet also exhibits a single band in the amide I region. However, this cannot be interpreted as evidence of chitin extraction, since the pellet still contains residual DES and entrapped proteins. The strong overlap between the absorption bands of these components masks the characteristic chitin signals, preventing a reliable identification of chitin in the unwashed material. To eliminate this interference, the pellet was washed three times with water, exploiting the insolubility of chitin in water and the hydrophobic nature of the DES, which readily separates from the aqueous phase. The washed pellet was subsequently dried and analysed by FTIR. As shown in **Figure S6.II**), the FTIR spectrum of the washed pellet closely matches that of the commercial chitin standard, displaying the characteristic broad band in the $3330\text{--}3260\text{ cm}^{-1}$ region attributed to O–H and N–H stretching vibrations involved in the extensive hydrogen-bonding network of α -chitin. The spectrum also exhibits the characteristic split amide I bands at approximately 1655 cm^{-1} and 1620 cm^{-1} (C=O stretching), the amide II band at approximately 1550 cm^{-1} (N–H bending coupled with C–N stretching), and the amide III band at approximately 1310 cm^{-1} (C–N stretching and N–H deformation). Additional characteristic chitin bands are observed in the $1150\text{--}1020\text{ cm}^{-1}$ region, corresponding to C–O–C and C–O stretching vibrations of the polysaccharide backbone [1–3]. These results confirm that chitin remained in the solid fraction throughout the extraction process and was not co-extracted by the DES. Together with the absence of minerals in the liquid phase (*cf.* Table S15 and Figure S5) and the limited protein co-extraction ($\sim 20\%$), these findings further demonstrate the high selectivity of the proposed extraction process towards astaxanthin recovery.

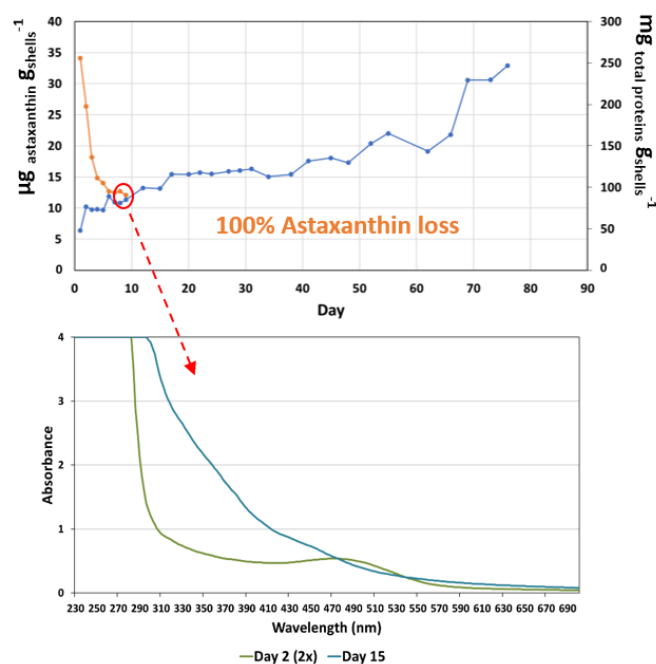


Figure S7. Thermal degradation of astaxanthin at 60 °C (in the dark) in acetic acid (control). I) Astaxanthin content progressively decreased, reaching complete loss by day 15. II) UV–Vis spectra confirm degradation by the disappearance of the characteristic visible absorption band (~482 nm), indicating disruption of the conjugated polyene system responsible for astaxanthin’s chromophore properties.

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