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Chlorophylls Extraction from Spinach Leaves Using Aqueous Solutions of Surface-Active Ionic Liquids

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Table S1. 2^2 factorial planning.

Experiment	χ_1	χ_2
1	−1	−1
2	1	−1
3	−1	1
4	1	1
5	−1.41	0
6	1.41	0
7	0	−1.41
8	0	1.41
9	0	0
10	0	0
11	0	0

Table S2. Independent variables coded levels used in factorial planning using [C_{16}py]Cl aqueous solutions.

Independent variables	Coded levels				
	Axial point −1.41	Factorial point −1	Central point 0	Factorial point 1	Axial point 1.41
CPC concentration (wt. %)	2.95	5.00	10.00	15.00	17.05
Solid-liquid ratio (R)	0.003	0.005	0.010	0.015	0.017

Table S3. Independent variables coded levels used in factorial planning using ethanol solutions.

Independent variables	Coded levels				
	Axial point −1.41	Factorial point −1	Central point 0	Factorial point 1	Axial point 1.41
Ethanol concentration (%)	58.85	65.00	80.00	95.00	101.15
Solid-liquid ratio (R)	0.003	0.005	0.010	0.015	0.017

Table S4. Surface-active ionic liquids used in this work, and their respective critical micellar concentrations (CMC) and IL concentration values (in mM) applied in chlorophyll extraction - IL screening.

Ionic liquid name	Abbreviation	CMC (mM)	[IL]* (mM)	REF.
Myristyltrimethylammonium bromide	$[N_{11114}]Br$	3. 75	297	[1]
Hexadecylpyridinium chloride	$[C_{16}py]Cl$	0.99	294	[2]
Cetylpyridium bromide	$[C_{16}py]Br$	0.33	260	[3]
1-methyl–3-tetradecylimidazolium chloride	$[C_{14}mim]Cl$	3.90	318	[4]
Hexadecyltrimethylammonium bromide	$[N_{16111}]Br$	0.91	274	[5]
Tributyltetradecylphosphonium chloride	$[P_{44414}]Cl$,	8.10	230	[6]

*which corresponds to a concentration of 10wt%

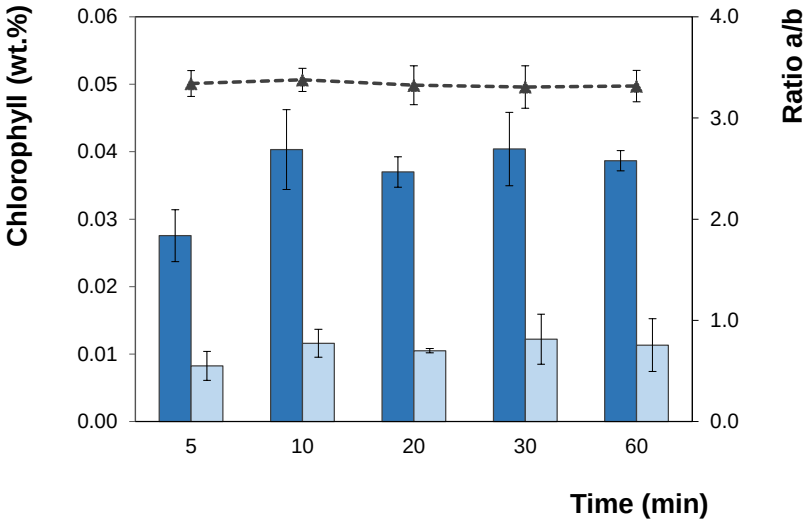

Figure S1. Yield of extracted chlorophyll *a* (■) and chlorophyll *b* (■) from spinach leaves using 10 wt.% $[C_{16}py]Cl$ solution in water and different extraction times (R=0.02; T=25°C) and chlorophyll *a*/*b* ratio (▲).

Table S5. Experimental data and response surface predicted values of the factorial planning using $[C_{16}py]Cl$ aqueous solutions.

			Chlorophyll *a* (wt. %)			Chlorophyll *b* (wt. %)			Chlorophyll *a*/*b* ratio		
	[IL] (wt. %)	R	Exp.	Pred.	relative error (%)	Exp.	Pred.	relative error (%)	Exp.	Pred.	relative error (%)
1	**5.0**	0.005	0.062	0.058	5.7	0.019	0.018	2.1	3.32	3.19	4.1
2	15.0	0.005	0.075	0.079	−4.6	0.018	0.019	−5.3	4.18	4.04	3.2
3	5.0	0.015	0.058	0.055	6.6	0.025	0.026	−3.7	2.33	2.05	12.4
4	15.0	0.015	0.084	0.087	−3.7	0.020	0.022	−11.5	4.28	3.99	6.7
5	3.0	0.010	0.058	0.063	−8.9	0.023	0.023	−0.1	2.52	2.74	−8.5
6	17.1	0.010	0.105	0.100	4.6	0.022	0.021	8.5	4.68	4.71	−0.7

7	10.0	0.003	0.056	0.056	0.1	0.017	0.017	0.1	3.29	3.32	−0.9
8	10.0	0.017	0.059	0.060	−0.7	0.027	0.025	7.0	2.23	2.48	−11.1
9	10.0	0.010	0.064	0.064	0.1	0.021	0.022	−1.5	3.00	2.93	2.3
10	10.0	0.010	0.064	0.064	0.7	0.020	0.022	−6.9	3.18	2.93	7.8
11	10.0	0.010	0.063	0.064	−0.8	0.023	0.022	7.2	2.72	2.93	−7.9

Nomenclature: [IL]: IL concentration; R: solid-liquid ratio; Exp.: experimental data; Pred.: predicted values.

Table S6. Regression coefficients of the predicted second-order polynomial model for the chlorophyll *a*, from factorial planning using [C_{16}py]Cl aqueous solutions. R-sqr=.95436; Adj:.90871.

	Regression coefficients	**Standard deviation**	**t-student**	**p-value**
Interception	0.071	0.01412	5.04383	0.003954
R	1.309	1.77788	0.73625	0.494649
R^2	−115.310	75.29066	−1.53154	0.186202
C	−0.006	0.00178	−3.19629	0.024095
C^2	0.000	0.00008	4.71753	0.005254
C x R	0.123	0.08910	1.38040	0.225986

Table S7. Regression coefficients of the predicted second-order polynomial model for the chlorophyll *b*, from factorial planning using [C_{16}py]Cl aqueous solutions. R-sqr=.78277; Adj:.56555.

	Regression coefficients	**Standard deviation**	**t-student**	**p-value**
Interception	0.0119	0.00617	1.92552	0.112133
R	1.2933	0.77723	1.66395	0.157007
R^2	−14.1957	32.91454	−0.43129	0.684220
C	0.0003	0.00078	0.36228	0.731949
C^2	0.0000	0.00003	0.04722	0.964165
C x R	−0.0478	0.03895	−1.22618	0.274724

Table S8. Regression coefficients of the predicted second-order polynomial model for the chlorophyll a/b ratio, from factorial planning using [C_{16}py]Cl aqueous solutions. R-sqr=.94078; Adj:.88156.

	Regression coefficients	**Standard deviation**	**t-student**	**p-value**
Interception	4.758	0.894	5.32284	0.003133
R	−157.089	112.594	−1.39519	0.221763
R^2	−579.551	4768.170	−0.12155	0.907992
C	−0.289	0.113	−2.56558	0.050305
C^2	0.016	0.005	3.42022	0.018834
C x R	10.919	5.643	1.93516	0.110756

Table S9. ANOVA results for the chlorophyll *a* extraction from factorial planning using [C_{16}py]Cl aqueous solutions.

	Chlorophyll *a*				
	Sum of squares	**Degrees of freedom**	**Mean square**	**F-value**	p-value
Regression	0.002056	5	0.000411	17.43268	0.003510
Residuals	0.000118	5	0.000024		
Total	0.002174				

Table S10. ANOVA results for the chlorophyll *b* extraction from factorial planning using [C16py]Cl aqueous solutions.

	Chlorophyll *b*				
	Sum of squares	**Degrees of freedom**	**Mean square**	**F-value**	p-value
Regression	0.000072	5	0.000014	4.731408	0.046614
Residuals	0.000015	5	0.000003		
Total	0.000087				

Table S11. ANOVA results for the chlorophyll a/b ratio extraction from factorial planning using [C16py]Cl aqueous solutions.

	Chlorophyll *b*				
	Sum of squares	**Degrees of freedom**	**Mean square**	**F-value**	p-value
Regression	6.350867	5	1.270173	17.17165	0.003634
Residuals	0.369846	5	0.073969		
Total	6.720713				

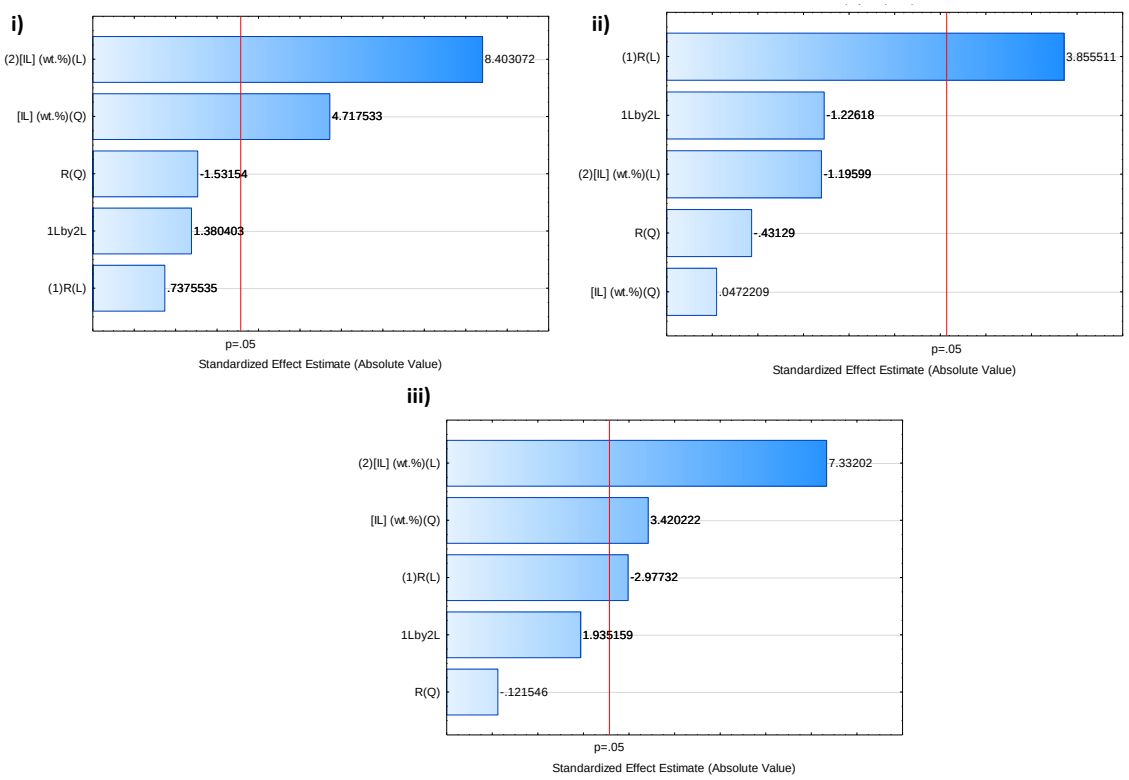

Figure S2. Pareto charts for the standardized main effects in the factorial planning using [C16py]Cl aqueous solutions for (i) chlorophyll *a*, (ii) chlorophyll *b* and (iii) chlorophyll *a*/*b* ratio. Vertical line indicates the statistical significance of the effects.

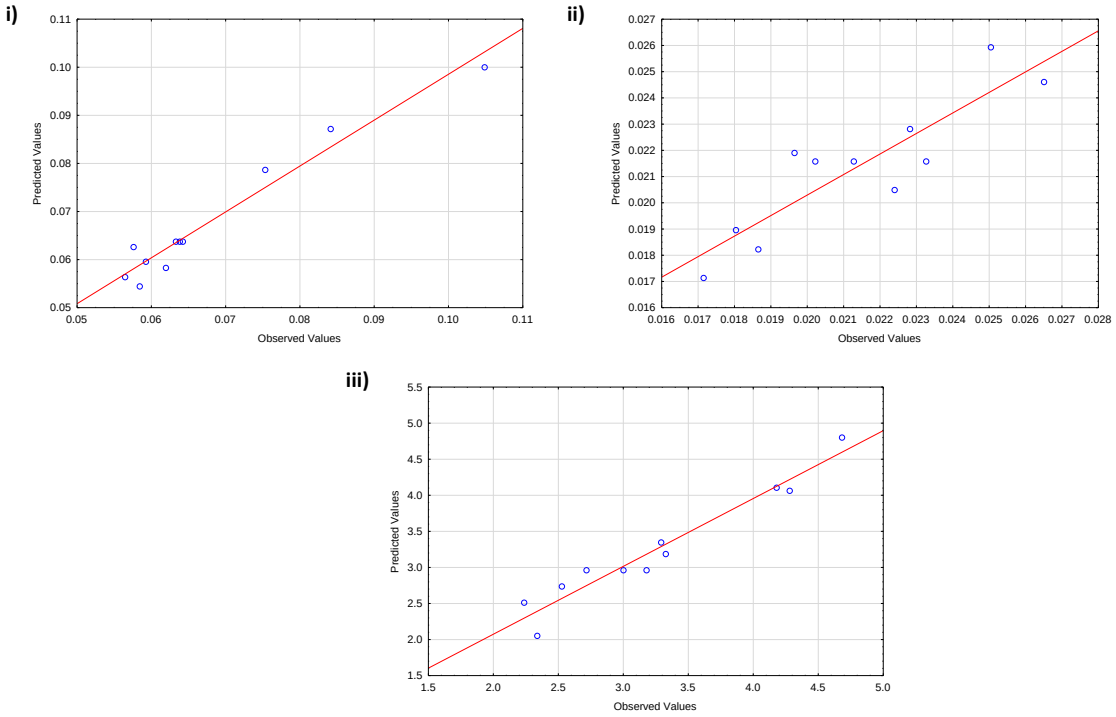

Figure S3. Observed values vs Predicted values in the factorial planning [C_{16}py]Cl aqueous solutions (i) chlorophyll *a*, (ii) chlorophyll *b* and (iii) chlorophyll *a*/*b* ratio.

Table S12. Experimental data and response surface predicted values of the factorial planning using ethanol aqueous solutions.

			Chlorophyll *a* (wt. %)			Chlorophyll *b* (wt. %)			Chlorophyll *a*/*b* ratio		
	[Ethanol] (wt. %)	**R**	**Exp.**	**Pred.**	**relative error (%)**	**Exp.**	**Pred.**	**relative error (%)**	**Exp.**	**Pred.**	**relative error (%)**
1	**65.0**	0.005	0.047	0.044	6.36	0.037	0.040	−6.62	1.25	1.02	18.32
2	95.0	0.005	0.099	0.092	6.58	0.028	0.031	−10.62	3.52	3.19	9.43
3	65.0	0.015	0.048	0.053	−12.23	0.032	0.032	1.86	1.48	1.67	−12.67
4	95.0	0.015	0.099	0.101	−2.34	0.027	0.027	0.33	3.72	3.80	−2.30
5	58.9	0.010	0.036	0.034	5.77	0.034	0.033	2.60	1.07	1.07	−0.39
6	100.0	0.010	0.099	0.102	−3.10	0.025	0.024	6.71	3.92	4.08	−4.02
7	80.0	0.003	0.065	0.071	−10.25	0.044	0.040	7.76	1.47	1.85	−25.22
8	80.0	0.017	0.090	0.084	6.58	0.031	0.032	−3.19	2.95	2.73	7.61
9	80.0	0.010	0.081	0.088	−8.84	0.025	0.028	−8.18	3.18	3.21	−0.84
10	80.0	0.010	0.092	0.088	3.52	0.028	0.028	0.12	3.32	3.21	3.18
11	80.0	0.010	0.093	0.088	4.63	0.029	0.028	6.14	3.15	3.21	−1.84

Nomenclature: [IL]: IL concentration; R: solid-liquid ratio; Exp.: Experimental data; Pred.: predicted values.

Table S13. Regression coefficients of the predicted second-order polynomial model for the chlorophyll *a*, from factorial planning using ethanol solutions. R-sqr=.96244; Adj:.92487.

	Regression coefficients	**Standard deviation**	**t-student**	**p-value**
Interception	−0.366	0.1000	−3.66058	0.014586
R	5.394	4.6169	1.16832	0.295349
R^2	−215.204	123.3467	−1.74471	0.141484

C	0.009	0.0023	3.87156	0.011742
C^2	0.000	0.0000	−3.23975	0.022956
C x R	−0.002	0.0484	−0.03839	0.970866

Table S14. Regression coefficients of the predicted second-order polynomial model for the chlorophyll *b*, from factorial planning using ethanol solutions. R-sqr=.92847; Adj:.85693.

	Regression coefficients	**Standard deviation**	**t-student**	**p-value**
Interception	0.0884	0.03852	2.29546	0.070169
R	−5.0694	1.77789	−2.85136	0.035767
R^2	172.1788	47.49857	3.62493	0.015141
C	−0.0006	0.00089	−0.64969	0.544554
C^2	0.0000	0.00001	0.25842	0.806382
C x R	0.0124	0.01862	0.66781	0.533836

Table S15. Regression coefficients of the predicted second-order polynomial model for the chlorophyll a/b ratio, from factorial planning using ethanol solutions. R-sqr=.98187; Adj:.96374.

	Regression coefficients	**Standard deviation**	**t-student**	**p-value**
Interception	−14.2	4.063	−3.48989	0.017472
R	449.4	187.524	2.39633	0.061901
R^2	−18901.7	5009.951	−3.77283	0.012985
C	0.3	0.094	3.17896	0.024567
C^2	0.0	0.001	−2.45765	0.057394
C x R	−0.1	1.964	−0.05355	0.959371

Table S16. ANOVA results for the chlorophyll *a* extraction from factorial planning using ethanol solutions.

Chlorophyll *a*					
	Sum of squares	**Degrees of freedom**	**Mean square**	**F-value**	p-value
Regression	0.005894	5	0.001179	15.05493	0.004913
Residuals	0.000392	5	0.000078		
Total	0.006286				

Table S17. ANOVA results for the chlorophyll *b* extraction from factorial planning using ethanol solutions.

Chlorophyll *b*					
	Sum of squares	**Degrees of freedom**	**Mean square**	**F-value**	p-value
Regression	0.000319	5	0.000064	9.005930	0.015353
Residuals	0.000035	5	0.000007		
Total	0.000354				

Table S18. ANOVA results for the chlorophyll *a/b* ratio extraction from factorial planning using ethanol solutions.

Chlorophyll *b*					
	Sum of squares	**Degrees of freedom**	**Mean square**	**F-value**	p-value
Regression	11.17708	5	2.235416	18.22878	0.003166

Residuals	0.61316	5	0.122631
Total	11.79024		

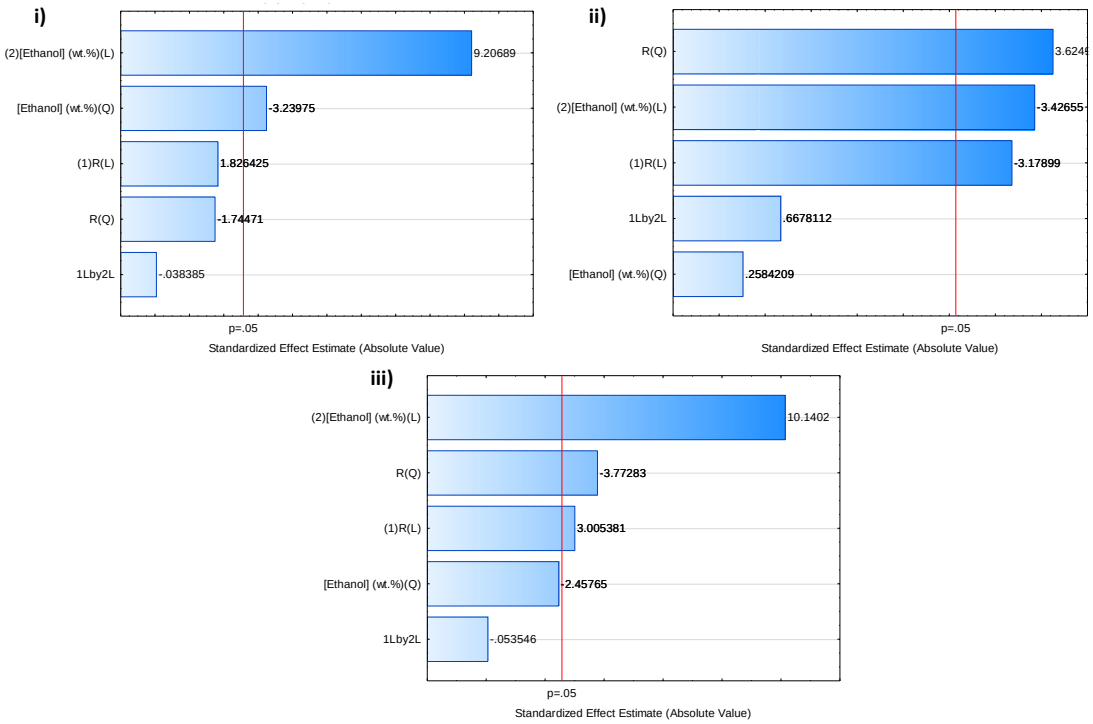

Figure S4. Pareto charts for the standardized main effects in the factorial planning using ethanol solutions for (i) chlorophyll *a*, (ii) chlorophyll *b* and (iii) chlorophyll *a*/*b* ratio. Vertical line indicates the statistical significance of the effects.

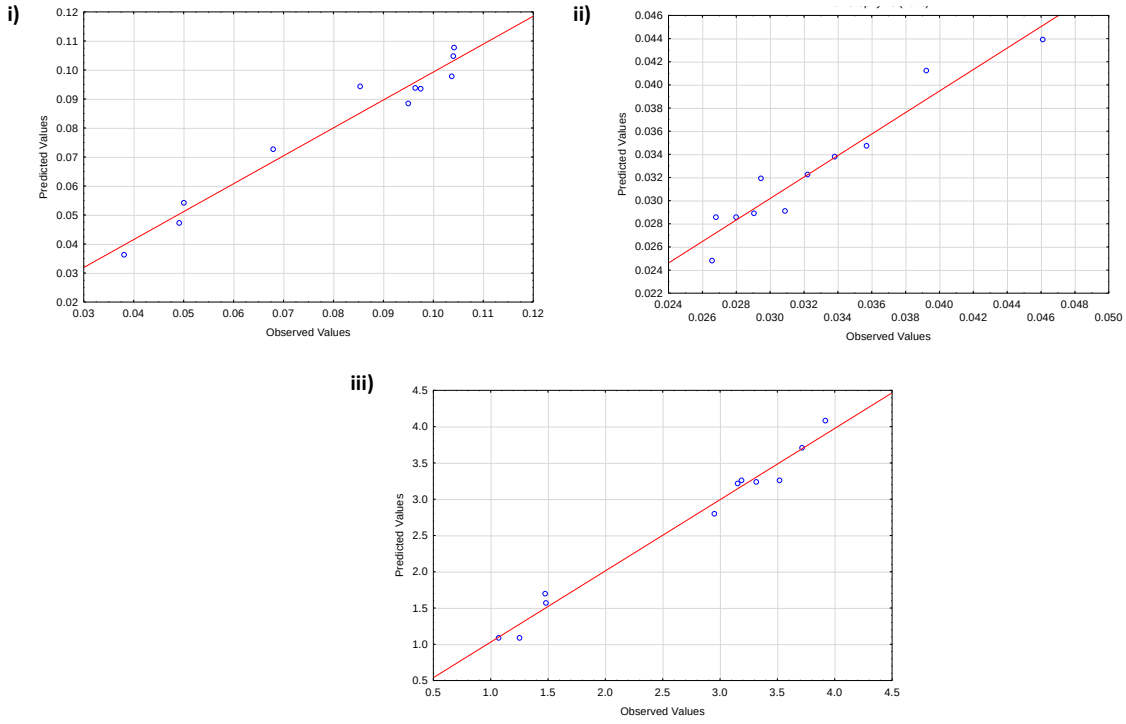

Figure S5. Observed values vs Predicted values in the factorial planning ethanol solutions (i) chlorophyll *a*, (ii) chlorophyll *b* and (iii) chlorophyll *a*/*b* ratio.

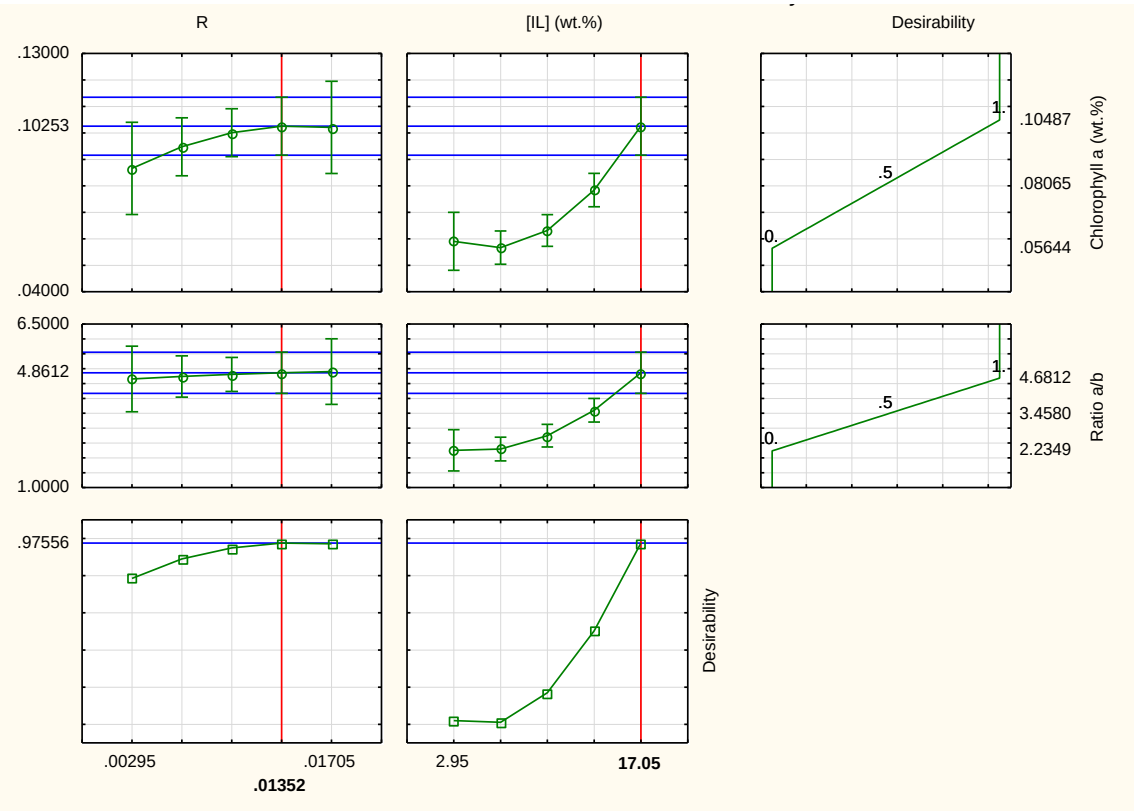

Figure S6. Profile of prediction of the optimal operating conditions to obtain the maximum yield of chlorophyll *a* and the chlorophylls a/b ratio.

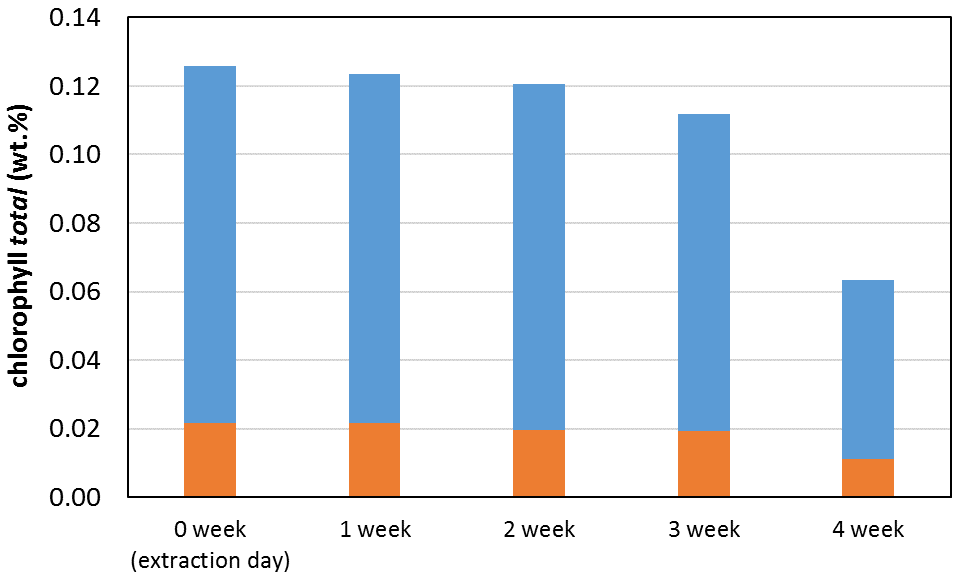

Figure S7. Profile of chlorophyll *a* (■) and *b* (■) content in the extract during the storage time, at 4°Cand protect of the light.

Table S19. Profile of chlorophyll *a* and *b* content in the extract during the storage time, at 4°Cand protect of the light.

Storage time	Chlorophyll *a* (wt. %)			Chlorophyll *b* (wt. %)		
0 week (extraction day)	0.104	±	0.009	0.022	±	0.004
1 week	0.102	±	0.010	0.022	±	0.004

2 week	0.101	±	0.011	0.020	±	0.007
3 week	0.093	±	0.010	0.019	±	0.007
4 week	0.053	±	0.010	0.011	±	0.008

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