

Supplementary Materials

Efficient extraction of the RuBisCO enzyme from
spinach leaves using aqueous solutions of
biocompatible ionic liquids

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Table S1. 2^3 factorial planning for each IL ([Ch]Cl and [Ch][Ac]).

| Experiment | 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 |

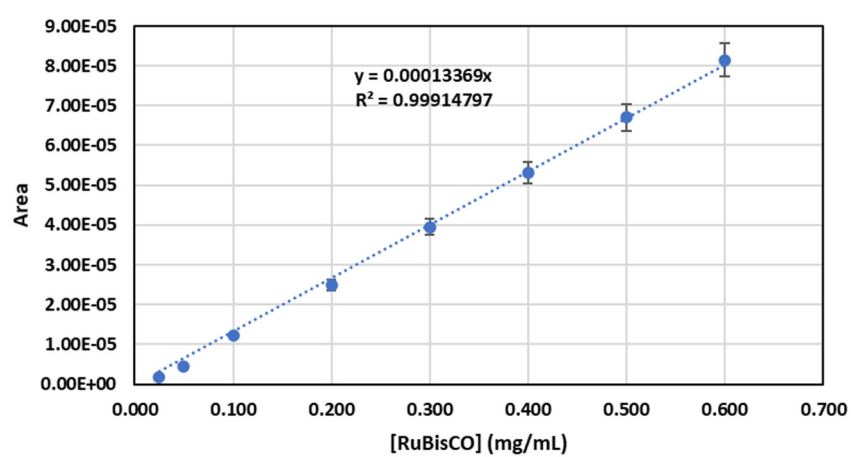


Figure S1. Calibration curve used to determine the concentration of RuBisCO in the samples. Equation: $\text{Area} = 1.3369 \times 10^{-4} [\text{RuBisCO}]$ and $R^2 = 0.9991$.

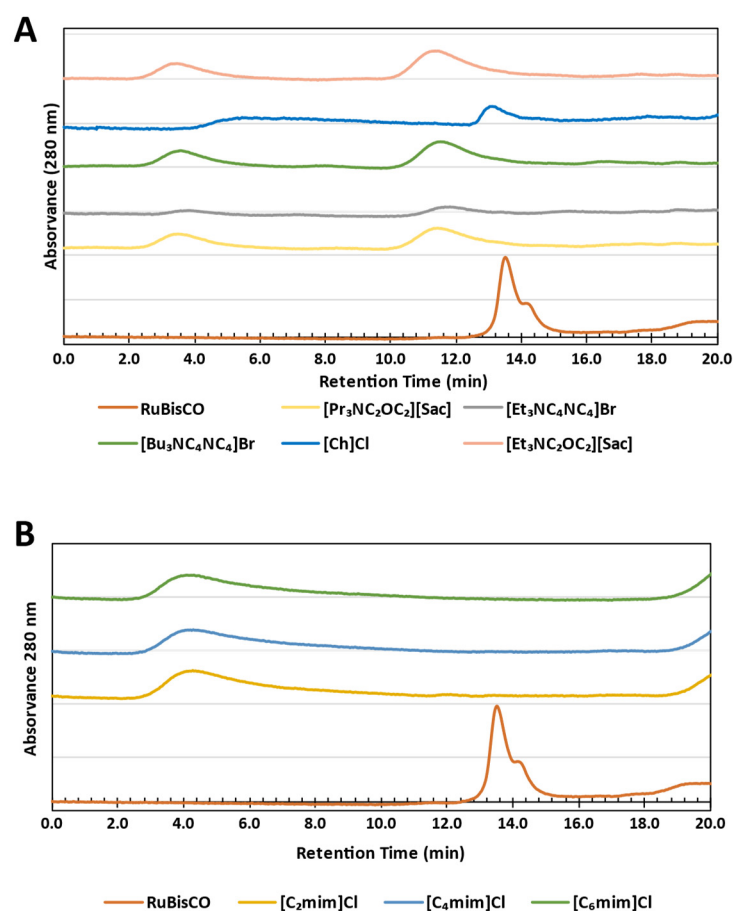


Figure S2. SE-HPLC spectra of aqueous solutions of biocompatible ILs (A) and imidazolium-based ILs (B) after RuBisCO's extraction from spinach leaves with an IL concentration of 3.3 mM. RuBisCO chromatogram presents 2 peaks. The peak with a retention time of ~13.4 min corresponds to the RuBisCO and the peak at ~14.3 min corresponds to impurities. The peaks with retention times of 4.0 and 11.6 min correspond to protein aggregates.

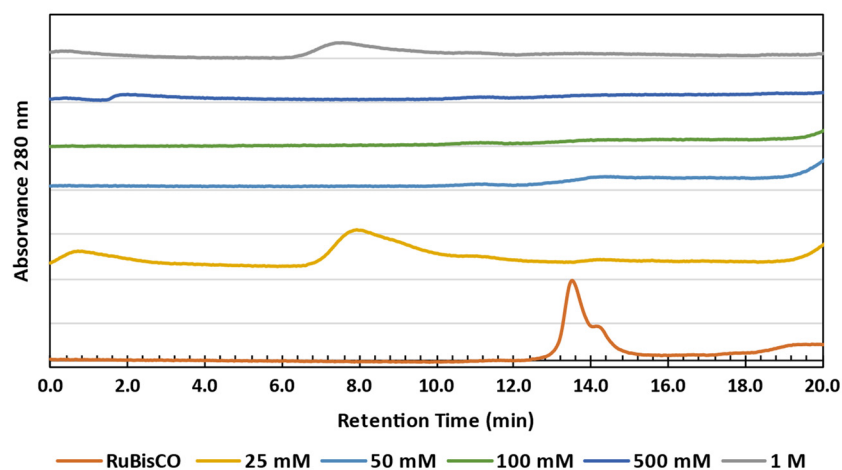


Figure S3. SE-HPLC spectra of aqueous solutions of [Ch][DHP] after RuBisCO's extraction from spinach leaves with different IL concentrations. RuBisCO chromatogram presents 2 peaks. The peak with a retention time of ~13.4 min corresponds to the RuBisCO and at ~14.3 min corresponds to impurities. The extracts with IL concentrations of 25 mM, 500 mM and 1 M present peaks with lower retention times (1.2, 2.0 and 8.0 min) corresponding to protein aggregates.

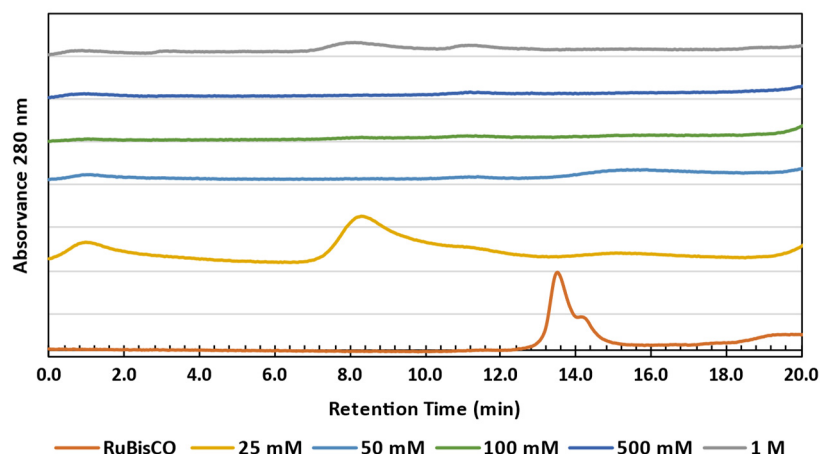


Figure S4. SE-HPLC spectra of aqueous solutions of [Ch][DHC] after RuBisCO's extraction from spinach leaves with different IL concentrations. RuBisCO chromatogram presents 2 peaks. The peak with a retention time of ~13.4 min corresponds to the RuBisCO and at ~14.3 min corresponds to impurities. The extract with IL concentrations of 25 mM and 1M present only peaks with lower retention times (1.2 and 8.0 min) corresponding to protein aggregates.

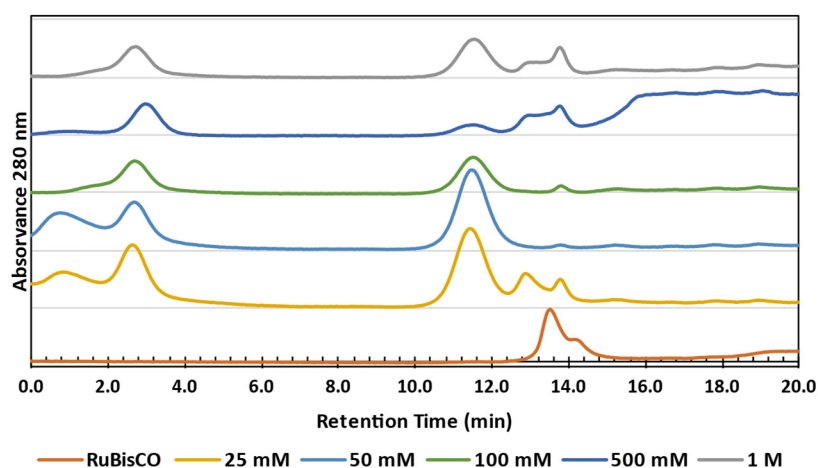


Figure S5. SE-HPLC spectra of aqueous solutions of [Ch]Cl after RuBisCO's extraction from spinach leaves with different IL concentrations. RuBisCO control chromatogram presents 2 peaks. The peak with a retention time of ~13.4 min corresponds to the RuBisCO and at ~14.3 min corresponds to impurities. The other peaks with lower retention times (1.2, 2.4, 11.6 and 12.6 min) correspond to protein aggregates.

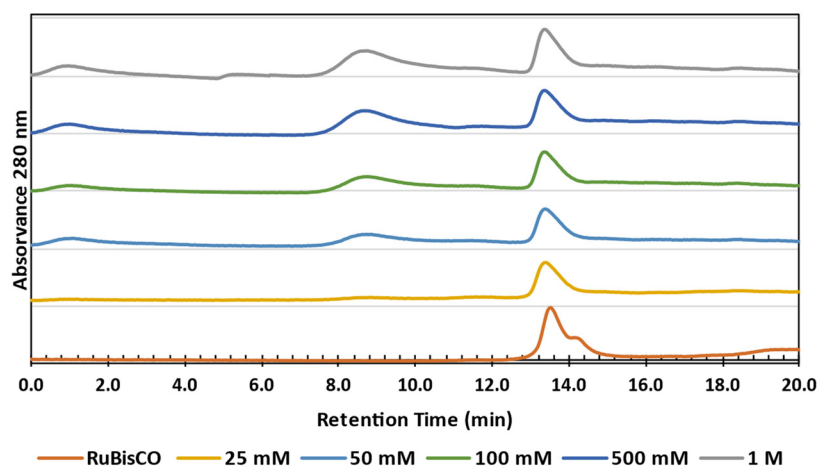


Figure S6. SE-HPLC spectra of aqueous solutions of [Ch][Ac] after RuBisCO's extraction from spinach leaves with different IL concentrations. RuBisCO chromatogram presents 2 peaks. The peak with a retention time of ~13.4 min corresponds to the RuBisCO and at ~14.3 min corresponds to impurities. All the extracts present a characteristic peak for RuBisCO. The peaks with a lower retention time (1.2 and 8.8 min) correspond to protein aggregates.

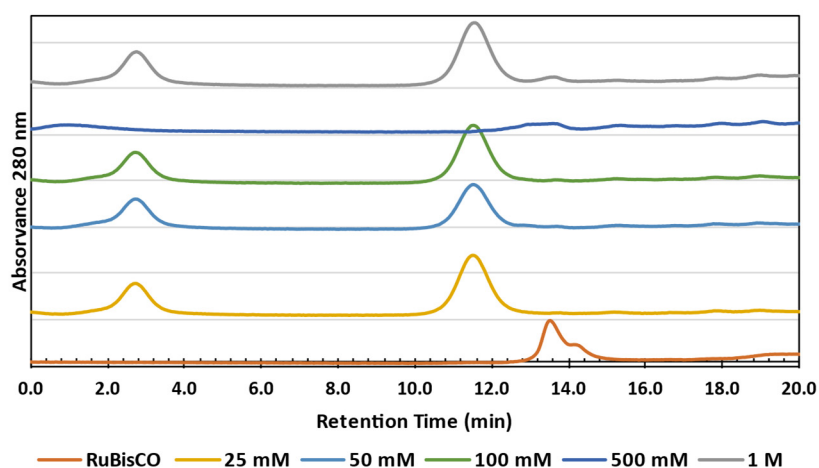


Figure S7. SE-HPLC spectra of aqueous solutions of [Ch]Br after RuBisCO's extraction from spinach leaves with different IL concentrations. RuBisCO chromatogram presents 2 peaks. The peak with a retention time of ~13.4 min corresponds to the RuBisCO and the peak at ~14.3 min corresponds to impurities. The peaks with lower retention times (2.8 and 11.6 min) correspond to protein aggregates.

Table S2. pH values of the IL aqueous solutions and of the extracts.

| [IL] (mM) | [Ch]Cl | | [Ch]Br | | [Ch][Ac] | | [Ch][DHC] | | [Ch][DHP] | |
|-----------|----------------------------|------------------------|----------------------------|------------------------|----------------------------|------------------------|----------------------------|------------------------|----------------------------|------------------------|
| | pH _{IL solutions} | pH _{extracts} | pH _{IL solutions} | pH _{extracts} | pH _{IL solutions} | pH _{extracts} | pH _{IL solutions} | pH _{extracts} | pH _{IL solutions} | pH _{extracts} |
| 25 | 5.29 | 6.45 | 5.70 | 6.39 | 6.16 | 6.30 | 3.77 | 4.13 | 3.47 | 5.37 |
| 50 | 3.97 | 6.23 | 4.07 | 6.29 | 6.03 | 6.12 | 3.72 | 3.93 | 3.43 | 5.07 |
| 100 | 4.35 | 6.28 | 5.28 | 6.26 | 5.99 | 6.03 | 3.69 | 3.82 | 3.42 | 4.42 |
| 500 | 5.70 | 6.23 | 5.12 | 6.15 | 6.03 | 6.03 | 3.66 | 3.72 | 3.41 | 3.65 |
| 1000 | 4.54 | 6.11 | 4.87 | 6.06 | 6.10 | 6.10 | 3.70 | 3.74 | 3.58 | 3.70 |

Table S3. Experimental data and response surface predicted values of the factorial planning for RuBisCOs' concentration using [Ch]Cl aqueous solutions.

| Nº | pH | S/L RATIO | C (M) | Predicted Results | Observed Results | Relative deviation (%) |
|----|------|--------------|-------|----------------------|---------------------|------------------------------|
| 1 | 4.50 | 0.05 | 0.80 | 0.516 | 0.519 | 0.530 |
| 2 | 9.50 | 0.05 | 0.80 | 0.526 | 0.581 | 9.49 |
| 3 | 4.50 | 0.15 | 0.80 | 1.25 | 1.34 | 6.74 |
| 4 | 9.50 | 0.15 | 0.80 | 1.37 | 1.37 | -0.036 |
| 5 | 4.50 | 0.05 | 2.2 | 0.410 | 0.370 | -10.9 |
| 6 | 9.50 | 0.05 | 2.2 | 0.541 | 0.410 | -31.9 |
| 7 | 4.50 | 0.15 | 2.2 | 1.12 | 1.03 | -9.36 |
| 8 | 9.50 | 0.15 | 2.2 | 1.37 | 1.33 | -3.29 |
| 9 | 2.80 | 0.10 | 1.5 | 0.703 | 0.709 | 0.892 |
| 10 | 11.2 | 0.10 | 1.5 | 0.921 | 0.973 | 5.31 |
| 11 | 7.00 | 0.02 | 1.5 | 0.189 | 0.237 | 20.2 |
| 12 | 7.00 | 0.18 | 1.5 | 1.50 | 1.51 | 0.673 |
| 13 | 7.00 | 0.10 | 0.32 | 1.05 | 0.940 | -11.4 |
| 14 | 7.00 | 0.10 | 2.7 | 0.956 | 1.12 | 14.8 |
| 15 | 7.00 | 0.10 | 1.5 | 0.854 | 0.898 | 4.90 |
| 16 | 7.00 | 0.10 | 1.5 | 0.854 | 0.901 | 5.21 |
| 17 | 7.00 | 0.10 | 1.5 | 0.854 | 0.744 | -14.9 |
| 18 | 7.00 | 0.10 | 1.5 | 0.854 | 0.777 | -9.98 |
| 19 | 7.00 | 0.10 | 1.5 | 0.854 | 0.905 | 5.59 |
| 20 | 7.00 | 0.10 | 1.5 | 0.854 | 0.890 | 4.04 |

Table S4. Experimental data and response surface predicted values of the factorial planning for RuBisCOs' extraction yield using [Ch]Cl aqueous solutions.

| Nº | pH | S/L RATIO | C (M) | Predicted Results | Observed Results | Relative deviation (%) |
|----|------|--------------|-------|----------------------|---------------------|------------------------------|
| 1 | 4.50 | 0.05 | 0.80 | 8.73 | 7.36 | -18.7 |
| 2 | 9.50 | 0.05 | 0.80 | 10.2 | 8.80 | -15.5 |
| 3 | 4.50 | 0.15 | 0.80 | 6.36 | 6.00 | -5.96 |
| 4 | 9.50 | 0.15 | 0.80 | 7.41 | 6.72 | -10.3 |
| 5 | 4.50 | 0.05 | 2.2 | 10.4 | 10.0 | -4.29 |
| 6 | 9.50 | 0.05 | 2.2 | 11.9 | 11.2 | -6.82 |
| 7 | 4.50 | 0.15 | 2.2 | 8.56 | 8.80 | 2.73 |
| 8 | 9.50 | 0.15 | 2.2 | 9.66 | 9.92 | 2.56 |
| 9 | 2.80 | 0.10 | 1.5 | 6.47 | 7.07 | 8.52 |
| 10 | 11.2 | 0.10 | 1.5 | 8.61 | 9.59 | 10.3 |
| 11 | 7.00 | 0.02 | 1.5 | 12.3 | 14.1 | 12.8 |
| 12 | 7.00 | 0.18 | 1.5 | 8.35 | 8.14 | -2.59 |
| 13 | 7.00 | 0.10 | 0.32 | 7.84 | 9.55 | 17.9 |
| 14 | 7.00 | 0.10 | 2.7 | 11.2 | 11.1 | -1.13 |
| 15 | 7.00 | 0.10 | 1.5 | 8.45 | 9.02 | 6.30 |
| 16 | 7.00 | 0.10 | 1.5 | 8.45 | 8.72 | 3.09 |
| 17 | 7.00 | 0.10 | 1.5 | 8.45 | 7.39 | -14.4 |
| 18 | 7.00 | 0.10 | 1.5 | 8.45 | 7.67 | -10.2 |
| 19 | 7.00 | 0.10 | 1.5 | 8.45 | 8.77 | 3.64 |
| 20 | 7.00 | 0.10 | 1.5 | 8.45 | 8.87 | 4.70 |

Table S5. Experimental data and response surface predicted values of the factorial planning for RuBisCOs' concentration using [Ch][Ac] aqueous solutions.

| Nº | pH | S/L RATIO | C (M) | Predicted Results | Observed Results | Relative deviation (%) |
|----|------|--------------|-------|----------------------|---------------------|------------------------------|
| 1 | 4.50 | 0.05 | 0.80 | 0.074 | 0.000 | |
| 2 | 9.50 | 0.05 | 0.80 | 0.412 | 0.482 | 14.4 |
| 3 | 4.50 | 0.15 | 0.80 | 0.260 | 0.000 | |
| 4 | 9.50 | 0.15 | 0.80 | 1.622 | 1.516 | -6.99 |
| 5 | 4.50 | 0.05 | 2.2 | 0.093 | 0.000 | |
| 6 | 9.50 | 0.05 | 2.2 | 0.427 | 0.488 | 12.5 |
| 7 | 4.50 | 0.15 | 2.2 | 0.268 | 0.000 | |
| 8 | 9.50 | 0.15 | 2.2 | 1.625 | 1.500 | -8.34 |
| 9 | 2.80 | 0.10 | 1.5 | -0.317 | 0.000 | |
| 10 | 11.2 | 0.10 | 1.5 | 1.109 | 1.073 | -3.36 |
| 11 | 7.00 | 0.02 | 1.5 | 0.074 | 0.000 | |
| 12 | 7.00 | 0.18 | 1.5 | 1.238 | 1.594 | 22.3 |
| 13 | 7.00 | 0.10 | 0.32 | 0.757 | 0.882 | 14.1 |
| 14 | 7.00 | 0.10 | 2.7 | 0.776 | 0.933 | 16.8 |
| 15 | 7.00 | 0.10 | 1.5 | 0.749 | 0.740 | -1.19 |
| 16 | 7.00 | 0.10 | 1.5 | 0.749 | 0.832 | 10.0 |
| 17 | 7.00 | 0.10 | 1.5 | 0.749 | 0.802 | 6.60 |
| 18 | 7.00 | 0.10 | 1.5 | 0.749 | 0.640 | -16.9 |
| 19 | 7.00 | 0.10 | 1.5 | 0.749 | 0.720 | -4.03 |
| 20 | 7.00 | 0.10 | 1.5 | 0.749 | 0.710 | -5.46 |

Table S6. Experimental data and response surface predicted values of the factorial planning for RuBisCOs' extraction yield using [Ch][Ac] aqueous solutions.

| Nº | pH | S/L RATIO | C (M) | Predicted Results | Observed Results | Relative deviation (%) |
|----|------|--------------|-------|----------------------|---------------------|------------------------------|
| 1 | 4.50 | 0.05 | 0.80 | 0.280 | 0.00 | |
| 2 | 9.50 | 0.05 | 0.80 | 7.95 | 8.79 | 9.56 |
| 3 | 4.50 | 0.15 | 0.80 | 2.18 | 0.00 | |
| 4 | 9.50 | 0.15 | 0.80 | 10.8 | 9.94 | -8.73 |
| 5 | 4.50 | 0.05 | 2.2 | 0.400 | 0.00 | |
| 6 | 9.50 | 0.05 | 2.2 | 8.09 | 9.00 | 10.2 |
| 7 | 4.50 | 0.15 | 2.2 | 2.11 | 0.00 | |
| 8 | 9.50 | 0.15 | 2.2 | 10.8 | 9.76 | -10.1 |
| 9 | 2.80 | 0.10 | 1.5 | -2.34 | 0.00 | |
| 10 | 11.2 | 0.10 | 1.5 | 11.4 | 10.8 | -5.10 |
| 11 | 7.00 | 0.02 | 1.5 | 1.75 | 0.500 | -250 |
| 12 | 7.00 | 0.18 | 1.5 | 5.60 | 8.65 | 35.3 |
| 13 | 7.00 | 0.10 | 0.32 | 8.06 | 8.93 | 9.73 |
| 14 | 7.00 | 0.10 | 2.7 | 8.11 | 9.04 | 10.3 |
| 15 | 7.00 | 0.10 | 1.5 | 7.14 | 7.43 | 3.96 |
| 16 | 7.00 | 0.10 | 1.5 | 7.14 | 6.98 | -2.28 |
| 17 | 7.00 | 0.10 | 1.5 | 7.14 | 7.69 | 7.18 |
| 18 | 7.00 | 0.10 | 1.5 | 7.14 | 6.29 | -13.4 |
| 19 | 7.00 | 0.10 | 1.5 | 7.14 | 7.22 | 1.11 |
| 20 | 7.00 | 0.10 | 1.5 | 7.14 | 6.90 | -3.43 |

Table S7. Regression coefficients of the predicted second-order polynomial model for the RuBisCOs' concentration from RSM using [Ch]Cl aqueous solutions, $R^2 = 0.95197$ and $r_{adj.} = 0.90875$.

| | Regression Coefficients | Standard deviation | t-student (10) | p-value |
|------------------------------------------|----------------------------|-----------------------|-------------------|---------|
| Interception | 0.3896 | 0.4504 | 0.8649 | 0.4074 |
| pH | 0.0097 | 0.0769 | 0.1261 | 0.9021 |
| pH ² | -0.0024 | 0.0044 | -0.5342 | 0.6049 |
| Solid-liquid Ratio | 6.583 | 3.491 | 1.886 | 0.0886 |
| Solid-liquid Ratio ² | -1.257 | 11.12 | -0.1130 | 0.9122 |
| IL Concentration | -0.4671 | 0.2534 | -1.843 | 0.0951 |
| IL Concentration ² | 0.1067 | 0.0567 | 1.880 | 0.0895 |
| pH x Solid-liquid Ratio | 0.2366 | 0.2983 | 0.7932 | 0.4461 |
| pH x IL Concentration | 0.0172 | 0.0213 | 0.8084 | 0.4377 |
| Solid-liquid Ratio x IL Concentration | -0.1247 | 1.065 | -0.1170 | 0.9092 |

Table S8. Effects of the variables in the second-order polynomial model for the extraction RuBisCO concentration using [Ch]Cl aqueous solutions.

| | Regression Coefficients | Standard deviation | t-student (10) | p-value |
|--------------------------------------------------|------------------------------------|-------------------------------|---------------------------|----------------|
| Interception | 0.8540 | 0.0430 | 19.85 | 0.0000 |
| pH | 0.1297 | 0.0571 | 2.271 | 0.0465 |
| pH² | -0.0297 | 0.0556 | -0.5342 | 0.6049 |
| Solid-liquid Ratio | 0.7801 | 0.0571 | 13.66 | 0.0000 |
| Solid-liquid Ratio² | -0.0063 | 0.0556 | -0.1130 | 0.9122 |
| IL Concentration | -0.0545 | 0.0571 | -0.9546 | 0.3623 |
| IL Concentration² | 0.1045 | 0.0556 | 1.880 | 0.0895 |
| pH x Solid-liquid Ratio | 0.0592 | 0.0746 | 0.7932 | 0.4461 |
| pH x IL Concentration | 0.0603 | 0.0746 | 0.8084 | 0.4377 |
| Solid-liquid Ratio x IL Concentration | -0.0087 | 0.0746 | -0.1170 | 0.9092 |

Table S9. Regression coefficients of the predicted second-order polynomial model for the RuBisCOs' extraction yield from RSM using [Ch]Cl aqueous solutions, $R^2 = 0.75851$ and $r_{adj.} = 0.54116$.

| | Regression Coefficients | Standard deviation | t-student (10) | p-value |
|--------------------------------------------------|------------------------------------|-------------------------------|---------------------------|----------------|
| Interception | 8.737 | 5.258 | 1.662 | 0.1276 |
| pH | 1.043 | 0.8981 | 1.161 | 0.2726 |
| pH² | -0.0518 | 0.0519 | -0.9967 | 0.3424 |
| Solid-liquid Ratio | -75.50 | 40.75 | -1.853 | 0.0936 |
| Solid-liquid Ratio² | 262.2 | 129.8 | 2.020 | 0.0710 |
| IL Concentration | -1.267 | 2.958 | -0.4283 | 0.6775 |
| IL Concentration² | 0.7607 | 0.6624 | 1.149 | 0.2775 |
| pH x Solid-liquid Ratio | -0.7645 | 3.483 | -0.2195 | 0.8306 |
| pH x IL Concentration | 0.0080 | 0.2487 | 0.0320 | 0.9751 |
| Solid-liquid Ratio x IL Concentration | 3.471 | 12.44 | 0.2791 | 0.7859 |

Table S10. Effects of the variables in the second-order polynomial model for the extraction yield of RuBisCO using [Ch]Cl aqueous solutions.

| | Regression Coefficients | Standard deviation | t-student (10) | p-value |
|---------------------------------------|-------------------------|--------------------|----------------|---------|
| Interception | 8.453 | 0.5021 | 16.83 | 0.0000 |
| pH | 1.269 | 0.6665 | 1.903 | 0.0862 |
| pH ² | -0.6470 | 0.6491 | -0.9967 | 0.3424 |
| Solid-liquid Ratio | -2.320 | 0.6665 | -3.481 | 0.0059 |
| Solid-liquid Ratio ² | 1.311 | 0.6491 | 2.020 | 0.0710 |
| IL Concentration | 1.985 | 0.6665 | 2.978 | 0.0139 |
| IL Concentration ² | 0.7455 | 0.6491 | 1.148 | 0.2775 |
| pH x Solid-liquid Ratio | -0.1911 | 0.8706 | -0.2195 | 0.8306 |
| pH x IL Concentration | 0.0279 | 0.8706 | 0.0320 | 0.9751 |
| Solid-liquid Ratio x IL Concentration | 0.2430 | 0.8706 | 0.2791 | 0.7859 |

Table S11. Regression coefficients of the predicted second-order polynomial model for the RuBisCOs' concentration from RSM using [Ch][Ac] aqueous solutions, R² = 0.90873 and r_{adj.} = 0.82659.

| | Regression Coefficients | Standard deviation | t-student (10) | p-value |
|---------------------------------------|-------------------------|--------------------|----------------|---------|
| Interception | -0.8088 | 0.9426 | -0.8580 | 0.4110 |
| pH | 0.2453 | 0.1610 | 1.524 | 0.1586 |
| pH ² | -0.0200 | 0.0093 | -2.144 | 0.0577 |
| Solid-liquid Ratio | -4.665 | 7.305 | -0.6386 | 0.5374 |
| Solid-liquid Ratio ² | -13.08 | 23.27 | -0.5620 | 0.5865 |
| IL Concentration | -0.0179 | 0.5303 | -0.0338 | 0.9737 |
| IL Concentration ² | 0.0128 | 0.1187 | 0.1079 | 0.9162 |
| pH x Solid-liquid Ratio | 2.046 | 0.6243 | 3.277 | 0.0083 |
| pH x IL Concentration | -0.0006 | 0.0446 | -0.0145 | 0.9887 |
| Solid-liquid Ratio x IL Concentration | -0.0793 | 2.230 | -0.0356 | 0.9723 |

Table S12. Effects of the variables in the second-order polynomial model for the RuBisCO concentration using [Ch][Ac] aqueous solutions.

| | Regression Coefficients | Standard deviation | t-student (10) | p-value |
|---------------------------------------|-------------------------|--------------------|----------------|---------|
| Interception | 0.7488 | 0.0900 | 8.318 | 0.0000 |
| pH | 0.8479 | 0.1195 | 7.097 | 0.0000 |
| pH ² | -0.2494 | 0.1164 | -2.144 | 0.0577 |
| Solid-liquid Ratio | 0.6922 | 0.1195 | 5.794 | 0.0002 |
| Solid-liquid Ratio ² | -0.0654 | 0.1164 | -0.5620 | 0.5865 |
| IL Concentration | 0.0113 | 0.1195 | 0.0948 | 0.9264 |
| IL Concentration ² | 0.0126 | 0.1164 | 0.1079 | 0.9162 |
| pH x Solid-liquid Ratio | 0.5115 | 0.1561 | 3.277 | 0.0083 |
| pH x IL Concentration | -0.0023 | 0.1561 | -0.0145 | 0.9887 |
| Solid-liquid Ratio x IL Concentration | -0.0056 | 0.1561 | -0.0356 | 0.9723 |

Table S13. Regression coefficients of the predicted second-order polynomial model for the RuBisCOs' extraction yield from RSM using [Ch][Ac] aqueous solutions, R² = 0.89709 and r_{adj} = 0.80447.

| | Regression Coefficients | Standard deviation | t-student (10) | p-value |
|---------------------------------------|-------------------------|--------------------|----------------|---------|
| Interception | -16.01 | 7.663 | -2.097 | 0.0624 |
| pH | 3.510 | 1.309 | 2.682 | 0.0230 |
| pH ² | -0.1482 | 0.0757 | -1.958 | 0.0787 |
| Solid-liquid Ratio | 109.5 | 59.39 | 1.844 | 0.0950 |
| Solid-liquid Ratio ² | -489.8 | 189.2 | -2.589 | 0.0270 |
| IL Concentration | -1.905 | 4.311 | -0.4418 | 0.6680 |
| IL Concentration ² | 0.6838 | 0.9653 | 0.7083 | 0.4949 |
| pH x Solid-liquid Ratio | 1.918 | 5.075 | 0.3779 | 0.7134 |
| pH x IL Concentration | 0.0025 | 0.3625 | 0.0069 | 0.9946 |
| Solid-liquid Ratio x IL Concentration | -1.407 | 18.13 | -0.0776 | 0.9396 |

Table S14. Effects of the variables in the second-order polynomial model for the extraction yield of RuBisCO using [Ch][Ac] aqueous solutions.

| | Regression Coefficients | Standard deviation | t-student (10) | p-value |
|---------------------------------------|-------------------------|--------------------|----------------|---------|
| Interception | 7.137 | 0.7318 | 9.752 | 0.0000 |
| pH | 8.158 | 0.9713 | 8.399 | 0.0000 |
| pH ² | -1.852 | 0.9460 | -1.958 | 0.0787 |
| Solid-liquid Ratio | 2.287 | 0.9713 | 2.354 | 0.0404 |
| Solid-liquid Ratio ² | -2.449 | 0.9460 | -2.589 | 0.0270 |
| IL Concentration | 0.0326 | 0.9713 | 0.0336 | 0.9739 |
| IL Concentration ² | 0.6701 | 0.9460 | 0.7083 | 0.4949 |
| pH x Solid-liquid Ratio | 0.4795 | 1.269 | 0.3779 | 0.7134 |
| pH x IL Concentration | 0.0088 | 1.269 | 0.0069 | 0.9946 |
| Solid-liquid Ratio x IL Concentration | -0.0985 | 1.269 | -0.0776 | 0.9396 |

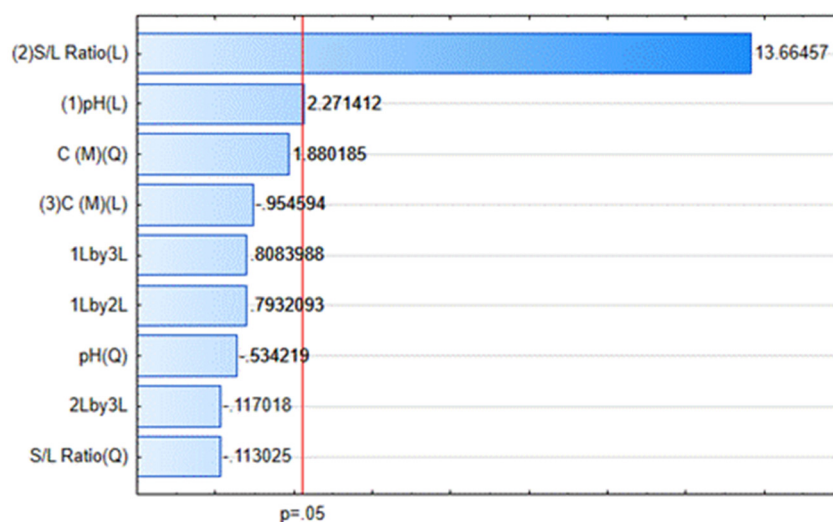


Figure S8. Pareto charts for the standardized main effects in the factorial planning with [Ch]Cl for RuBisCOs' concentration. The vertical line indicates the statistical significance of the effects.

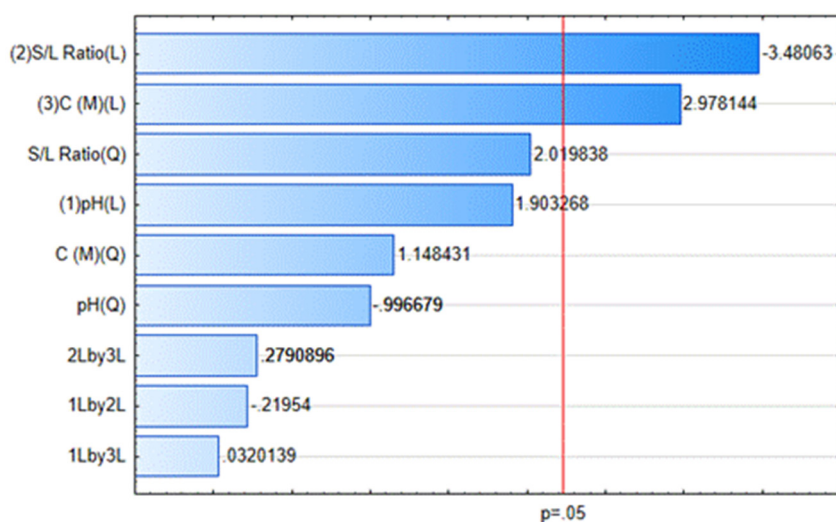


Figure S9. Pareto charts for the standardized main effects in the factorial planning with [Ch]Cl for RuBisCOs' extraction yield. The vertical line indicates the statistical significance of the effects.

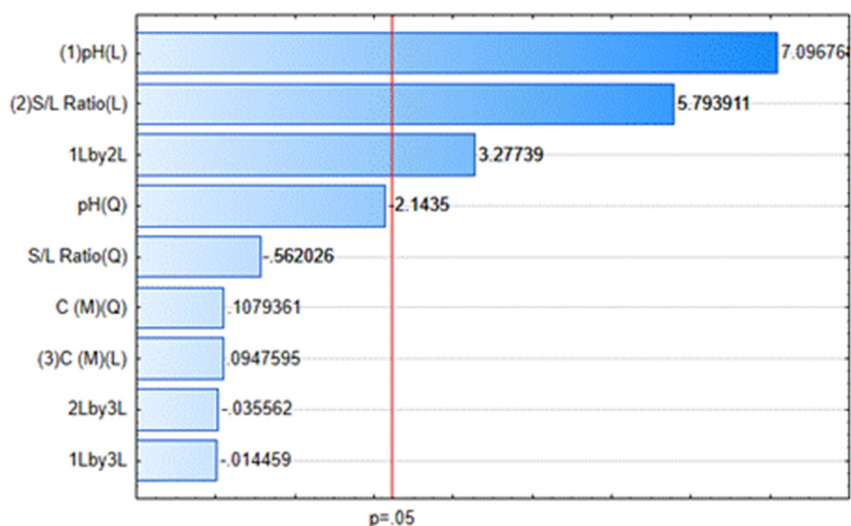


Figure S10. Pareto charts for the standardized main effects in the factorial planning with [Ch][Ac] for RuBisCOs' concentration. The vertical line indicates the statistical significance of the effects.

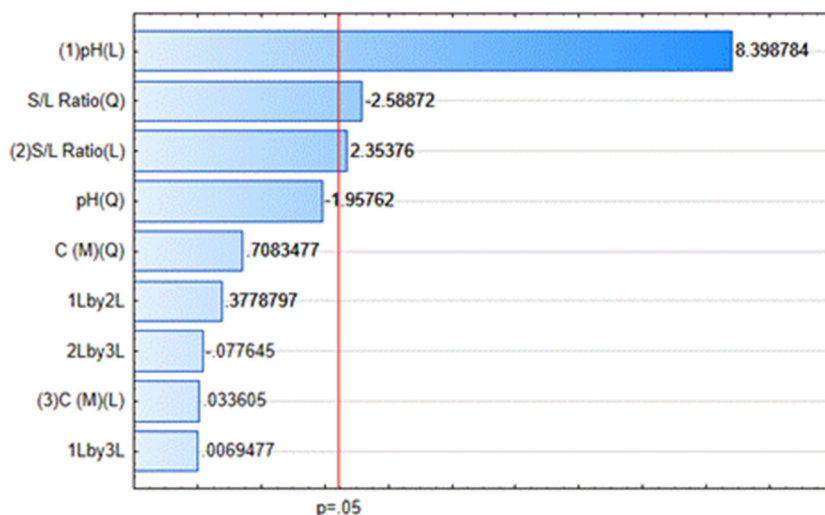


Figure S11. Pareto charts for the standardized main effects in the factorial planning with [Ch][Ac] for RuBisCOs' extraction yield. The vertical line indicates the statistical significance of the effects.

Table S15. ANOVA data for RuBisCO concentration when using [Ch]Cl aqueous solutions.

| | Sums of squares | Degrees of freedom | Mean square | F-value | p-value |
|------------|-----------------|--------------------|-------------|---------|----------|
| Regression | 2.20 | 9.00 | 0.2450 | 22.02 | 0.000019 |
| Residuals | 0.11 | 10.0 | 0.0110 | | |
| Total | 2.32 | | | | |

Table S16. ANOVA data for the extraction yield of RuBisCO when using [Ch]Cl aqueous solutions.

| | Sums of squares | Degrees of freedom | Mean square | F-value | p-value |
|------------|-----------------|--------------------|-------------|---------|---------|
| Regression | 47.61 | 9.00 | 5.291 | 3.490 | 0.0322 |
| Residuals | 15.16 | 10.0 | 1.516 | | |
| Total | 62.77 | | | | |

Table S17. ANOVA data for RuBisCO concentration when using [Ch][Ac] aqueous solutions.

| | Sums of squares | Degrees of freedom | Mean square | F-value | p-value |
|------------|-----------------|--------------------|-------------|---------|---------|
| Regression | 4.850 | 9.00 | 0.5400 | 11.06 | 0.00 |
| Residuals | 0.490 | 10.0 | 0.0500 | | |
| Total | 5.340 | | | | |

Table S18. ANOVA data for the extraction yield of RuBisCO when using [Ch][Ac] aqueous solutions.

| | Sums of squares | of Degrees of freedom | Mean square | F-value | p-value |
|------------|-----------------|-----------------------|-------------|---------|---------|
| Regression | 280.7 | 9.00 | 31.19 | 9.686 | 0.0007 |
| Residuals | 32.20 | 10.0 | 3.220 | | |
| Total | 312.9 | | | | |

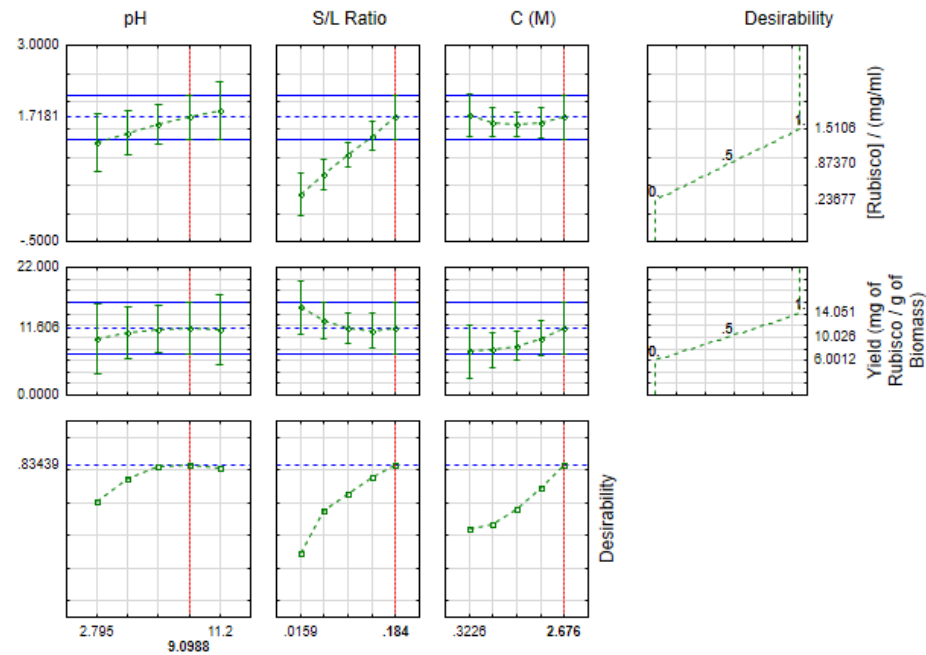


Figure S12. Profiles for predicted values and desirability in the factorial planning for both dependent variables with [Ch]Cl aqueous solutions.

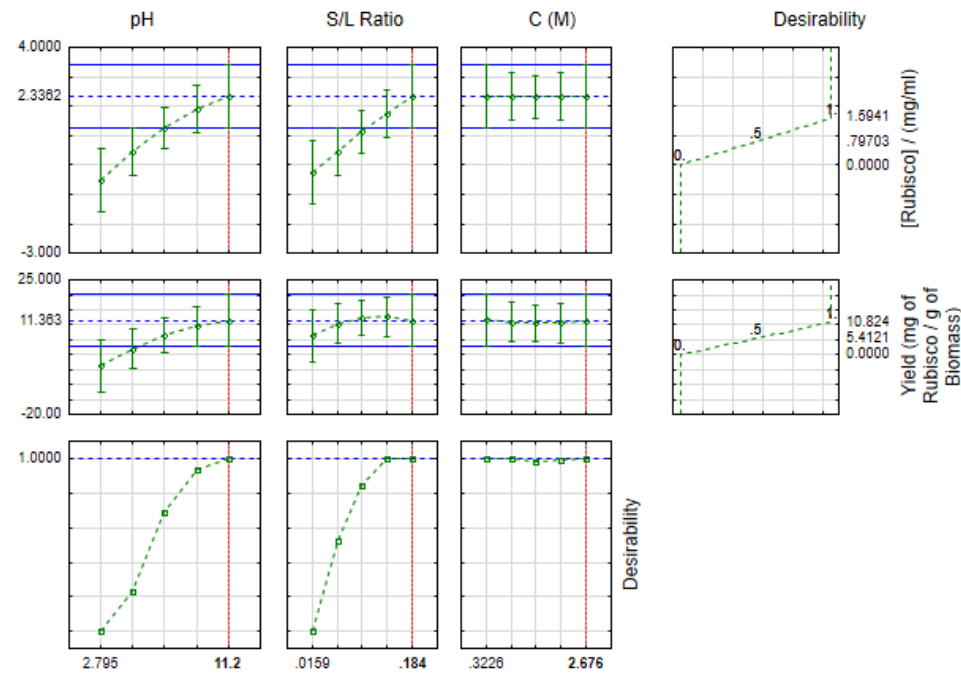


Figure S13. Profiles for predicted values and desirability in the factorial planning for both dependent variables with [Ch][Ac] aqueous solutions.