

Supplementary Materials: Extraction of Carotenoids from Pumpkin (*Cucurbita moschata*) and Spinach (*Spinacia oleracea*) Using Environmentally Friendly Deep Eutectic Solvents (DESs)

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Table S1. pH and density measurements of the DESs used in this study.

DES ¹	Molar Ratio (HBA:HBD) ²	pH	Density (g/ml)	Physical State at 5°C	Physical State at -18°C
M1ACA1	1:1	1.62	0.9392	Liquid	Liquid
M1ACA2	1:2	0.75	0.9540	Liquid	Liquid
M1ACA3	1:3	0.21	0.9670	Liquid	Solid
M1ACA4	1:4	0.37	0.9770	Liquid	Liquid
M1PRA1	1:1	1.76	0.9136	Liquid	Liquid
M1PRA2	1:2	1.16	0.9389	Liquid	Liquid
M1PRA3	1:3	0.68	0.9273	Liquid	Liquid
M1PRA4	1:4	0.46	0.9354	Liquid	Liquid
M1BTA1	1:1	1.01	0.9234	Liquid	Liquid
M1BTA2	1:2	0.83	0.9370	Liquid	Liquid
M1BTA3	1:3	0.67	0.9356	Liquid	Liquid
M1BTA4	1:4	0.42	0.9517	Liquid	Liquid

¹ DES: deep eutectic solvent, M: L-menthol, ACA: acetic acid, PRA: propionic acid, BTA: butyric acid. ² HBA: hydrogen bond acceptor, HBD: hydrogen bond donor

Table S2. Equations and correlation coefficients of calibration curves for UV/visible spectrophotometer analysis of β -carotene dissolved in DESs used in this study. y = β -carotene concentration (ppm) and x = UV absorbance reading at λ = 450 nm.

DES ¹	Calibration Curve Equation	R ²
M1ACA1	$y = 87.87346x - 0.28120$	0.99956
M1ACA2	$y = 91.12573x + 0.71360$	0.99555
M1ACA3	$y = 86.98367x + 0.19003$	0.99857
M1ACA4	$y = 85.72551x - 0.33361$	0.99913
M1PRA1	$y = 96.61229x + 4.71845$	0.99607
M1PRA2	$y = 135.50036x + 4.33645$	0.99728
M1PRA3	$y = 99.47225x + 1.58989$	0.99970
M1PRA4	$y = 95.52686x + 2.49283$	0.99970
M1BTA1	$y = 70.35002x + 0.60399$	0.99967
M1BTA2	$y = 131.59999x + 0.04304$	0.99621
M1BTA3	$y = 106.61199x + 0.76638$	0.99895
M1BTA4	$y = 114.77158x + 0.04332$	0.99909

¹ DES: deep eutectic solvent, M: L-menthol, ACA: acetic acid, PRA: propionic acid, BTA: butyric acid.

Table S3. The ANOVA evaluation of the second-order model equation derived for $\mu\text{g-}\beta\text{-carotene/gr-pumpkin}$ results of MMAE of $\beta\text{-carotene}$ from the pumpkin samples using M1ACA1, M1ACA2, M1ACA3 and M1ACA4.

Source	Sum of Squares	df	Mean Square	F Value	<i>p</i> -value	
Model	0.3313	5	0.0663	53.8006	<0.0001	significant
X ₁ -Acetic Acid Molar Ratio	0.1332	1	0.1332	108.1692	<0.0001	
X ₂ -Mixing Time	0.0003	1	0.0003	0.2756	0.6111	
X ₁ ²	0.1489	1	0.1489	120.9452	<0.0001	
X ₂ ²	0.0005	1	0.0005	0.3754	0.5538	
X ₁ .X ₂	0.0016	1	0.0016	1.3120	0.2787	
Residual	0.0123	10	0.0012			
Cor Total	0.3436	15				

Table S4. The ANOVA evaluation of the second-order model equation derived for $\mu\text{g-}\beta\text{-carotene/gr-pumpkin}$ results of MMAE of $\beta\text{-carotene}$ from the pumpkin samples using M1PRA1, M1PRA2, M1PRA3 and M1PRA4.

Source	Sum of Squares	df	Mean Square	F Value	<i>p</i> -value	
Model	81.0757	5	16.2151	18.1147	0.0001	significant
X ₁ -Propionic Acid Molar Ratio	10.5678	1	10.5678	11.8058	0.0064	
X ₂ -Mixing Time	5.805	1	5.805	6.485	0.0290	
X ₁ ²	50.206	1	50.206	56.0876	<0.0001	
X ₂ ²	0.0013	1	0.0013	0.0015	0.9700	
X ₁ .X ₂	9.4216	1	9.4216	10.5254	0.0088	
Residual	8.9514	10	0.8951			
Cor Total	90.0271	15				

Table S5. The ANOVA evaluation of the second-order model equation derived for $\mu\text{g-}\beta\text{-Carotene/gr-spinach}$ results of MMAE of $\beta\text{-Carotene}$ from spinach samples using M1ACA1, M1ACA2, M1ACA3 and M1ACA4.

Source	Sum of Squares	df	Mean Square	F Value	p-value	
Model	254.995	5	50.9990	1.3011	0.3372	not significant
X ₁ -Acetic Acid Molar Ratio	84.8315	1	84.8315	2.1643	0.1720	
X ₂ -Mixing Time	0.2017	1	0.2017	0.0051	0.9442	
X ₁ ²	73.6662	1	73.6662	1.8794	0.2004	
X ₂ ²	30.0496	1	30.0496	0.7667	0.4018	
X ₁ .X ₂	38.8038	1	38.8038	0.9900	0.3432	
Residual	391.9595	10	39.1959			
Cor Total	646.9545	15				

Table S6. The ANOVA evaluation of the second-order model equation derived for $\mu\text{g-}\beta\text{-carotene/gr-spinach}$ results of MMAE of $\beta\text{-carotene}$ from the spinach samples using M1PRA1, M1PRA2, M1PRA3 and M1PRA4.

Source	Sum of Squares	df	Mean Square	F Value	p-value	
Model	448.0706	5	89.6141	7.2392	0.0041	significant
X ₁ -Propionic Acid Molar Ratio	352.9684	1	352.9684	28.5136	0.0003	
X ₂ -Mixing Time	26.4831	1	26.4831	2.1394	0.1743	
X ₁ ²	78.6706	1	78.6706	6.3552	0.0303	
X ₂ ²	6.7925	1	6.7925	0.5487	0.4759	
X ₁ .X ₂	11.1946	1	11.1946	0.9043	0.3640	
Residual	123.7895	10	12.3789			
Cor Total	571.8601	15				

Table S7. The ANOVA evaluation of the second-order model equation derived for $\mu\text{g-}\beta\text{-carotene/gr-spinach}$ results of MMAE of $\beta\text{-carotene}$ from the spinach samples using M1BTA1, M1BTA2, M1BTA3 and M1BTA4.

Source	Sum of Squares	df	Mean Square	F Value	<i>p</i> -value	
Model	29.4059	5	5.8812	6.652	0.0056	significant
X ₁ -Butyric Acid Molar Ratio	1.4329	1	1.4329	1.6207	0.2318	
X ₂ -Mixing Time	21.6056	1	21.6056	24.4372	0.0006	
X ₁ ²	0.0813	1	0.0813	0.0920	0.7679	
X ₂ ²	0.6147	1	0.6147	0.6952	0.4239	
X ₁ .X ₂	1.27	1	1.2700	1.4364	0.2584	
Residual	8.8413	10	0.8841			
Cor Total	38.2471	15				

Table S8. The ANOVA evaluation of the second-order model equation derived for $\mu\text{g-}\beta\text{-carotene/gr-pumpkin}$ results of HAE of $\beta\text{-carotene}$ from the pumpkin samples using M1ACA1, M1ACA2, M1ACA3 and M1ACA4.

Source	Sum of Squares	df	Mean Square	F Value	<i>p</i> -value	
Model	0.6506	9	0.0723	22.4605	<0.0001	significant
X ₁ -Acetic Acid Molar Ratio	0.1595	1	0.1595	49.5466	<0.0001	
X ₂ -Homogenization Time	0.0344	1	0.0344	10.6947	0.0023	
X ₃ -Homogenization Speed	0.0414	1	0.0414	12.8656	0.0009	
X ₁ ²	0.3056	1	0.3056	94.9537	<0.0001	
X ₂ ²	0.0027	1	0.0027	0.8482	0.3629	
X ₃ ²	0.0032	1	0.0032	1.0041	0.3227	
X ₁ .X ₂	0.0002	1	0.0002	0.0641	0.8014	
X ₁ .X ₃	0.0330	1	0.0330	10.2595	0.0028	
X ₂ .X ₃	0.0074	1	0.0074	2.3029	0.1374	
Residual	0.1223	38	0.0032			
Cor Total	0.7729	47				

Table S9. The ANOVA evaluation of the second-order model equation derived for $\mu\text{g-}\beta\text{-carotene/gr-pumpkin}$ results of HAE of $\beta\text{-carotene}$ from the pumpkin samples using M1PRA1, M1PRA2, M1PRA3 and M1PRA4.

Source	Sum of Squares	df	Mean Square	F Value	p-value	
Model	142.4865	9	15.8318	10.1010	<0.0001	significant
X ₁ -Propionic Acid Molar Ratio	20.1919	1	20.1919	12.8828	0.0009	
X ₂ -Homogenization Time	46.2285	1	46.2285	29.4945	<0.0001	
X ₃ -Homogenization Speed	10.0320	1	10.0320	6.4006	0.0157	
X ₁ ²	19.5545	1	19.5545	12.4761	0.0011	
X ₂ ²	4.3344	1	4.3344	2.7654	0.1045	
X ₃ ²	2.2528	1	2.2528	1.4373	0.2380	
X ₁ .X ₂	3.2402	1	3.2402	2.0673	0.1587	
X ₁ .X ₃	19.4678	1	19.4678	12.4208	0.0011	
X ₂ .X ₃	3.1114	1	3.1114	1.9851	0.1670	
Residual	59.5596	38	1.5674			
Cor Total	202.0462	47				

Table S10. The ANOVA evaluation of the second-order model equation derived for $\mu\text{g-}\beta\text{-carotene/gr-spinach}$ results of HAE of $\beta\text{-carotene}$ from the spinach samples using M1ACA1, M1ACA2, M1ACA3 and M1ACA4.

Source	Sum of Squares	df	Mean Square	F Value	p-value	
Model	292.2342	9	32.4705	1.5812	0.1561	not significant
X ₁ -Acetic Acid Molar Ratio	95.6487	1	95.6487	4.6578	0.0373	
X ₂ -Homogenization Time	1.4957	1	1.4957	0.0728	0.7887	
X ₃ -Homogenization Speed	16.0399	1	16.0399	0.7811	0.3824	
X ₁ ²	14.4866	1	14.4866	0.7055	0.4062	
X ₂ ²	0.7864	1	0.7864	0.0383	0.8459	
X ₃ ²	60.1572	1	60.1572	2.9295	0.0951	
X ₁ .X ₂	77.9472	1	77.9472	3.7958	0.0588	
X ₁ .X ₃	4.8567	1	4.8567	0.2365	0.6295	
X ₂ .X ₃	0.0379	1	0.0379	0.0018	0.9659	
Residual	780.3381	38	20.5352			
Cor Total	1072.5723	47				

Table S11. The ANOVA evaluation of the second-order model equation derived for $\mu\text{g-}\beta\text{-carotene/gr-spinach}$ results of HAE of $\beta\text{-carotene}$ from the spinach samples using M1PRA1, M1PRA2, M1PRA3 and M1PRA4.

Source	Sum of Squares	df	Mean Square	F Value	<i>p</i> -value	
Model	782.8441	9	86.9827	7.9800	<0.0001	significant
X ₁ -Propionic Acid Molar Ratio	541.3456	1	541.3456	49.6642	<0.0001	
X ₂ -Homogenization Time	15.1182	1	15.1182	1.3870	0.2462	
X ₃ -Homogenization Speed	10.3061	1	10.3061	0.9455	0.3370	
X ₁ ²	157.1757	1	157.1757	14.4196	0.0005	
X ₂ ²	36.4601	1	36.4601	3.3449	0.0753	
X ₃ ²	20.5517	1	20.5517	1.8855	0.1778	
X ₁ .X ₂	14.9703	1	14.9703	1.3734	0.2485	
X ₁ .X ₃	12.9992	1	12.9992	1.1926	0.2817	
X ₂ .X ₃	26.8730	1	26.8730	2.4654	0.1247	
Residual	414.2047	38	10.9001			
Cor Total	1197.0488	47				

Table S12. The ANOVA evaluation of the second-order model equation derived for $\mu\text{g-}\beta\text{-carotene/gr-spinach}$ results of HAE of $\beta\text{-carotene}$ from the spinach samples using M1BTA1, M1BTA2, M1BTA3 and M1BTA4.

Source	Sum of Squares	df	Mean Square	F Value	<i>p</i> -value	
Model	166.1997	9	18.4666	3.7719	0.0018	significant
X ₁ -Butyric Acid Molar Ratio	77.8182	1	77.8182	15.8946	0.0003	
X ₂ -Homogenization Time	21.2588	1	21.2588	4.3422	0.0440	
X ₃ -Homogenization Speed	20.3191	1	20.3191	4.1502	0.0486	
X ₁ ²	0.6612	1	0.6612	0.1351	0.7153	
X ₂ ²	17.3040	1	17.3040	3.5344	0.0678	
X ₃ ²	5.7536	1	5.7536	1.1752	0.2852	
X ₁ .X ₂	3.7374	1	3.7374	0.7634	0.3878	
X ₁ .X ₃	0.1027	1	0.1027	0.0210	0.8856	
X ₂ .X ₃	22.1995	1	22.1995	4.5343	0.0398	
Residual	186.0437	38	4.8959			
Cor Total	352.2434	47				