

Methodological Optimization of Supercritical Fluid Extraction of Valuable Bioactive Compounds from the Acidophilic Microalga *Coccomyxa onubensis*

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Supplementary material - Table S1. Regression coefficients and p-Value for Extraction yield, Lutein purity, and Lutein recovery in their original units and statistics for the fit obtained by multiple linear regression.

Terms of model	Extraction Yield (%, w/w)		Lutein purity (mg/g extract)		Lutein recovery (%, w/w)	
	Estimate	p-Value	Estimate	p-Value	Estimate	p-Value
Constant	-16.1420		24.9590		-23.3342	
A: T.	0.3252	0.0244*	-0.1472	0.0113*	0.5381	0.0010*
B: P	0.4188	0.0083*	-0.7977	0.0700	0.7420	0.2454
C: EtOH %	0.4390	0.0000*	0.2390	0.5327	0.7138	0.0005*
T × Temp.	-0.0028	0.0166*	0.0009	0.8011	-0.0091	0.2630
T × P	0.0004	0.6926	0.0025	0.5874	0.0092	0.3648
T × EtOH %	-0.0011	0.1291	0.0055	0.0867	0.0270	0.0046*
P × P	-0.0048	0.0193*	0.0086	0.2082	-0.0129	0.3632
P × EtOH %	-0.0047	0.0021*	0.0050	0.2030	-0.0001	0.9898
EtOH % × EtOH %	0.0006	0.2798	-0.0138	0.0013*	-0.0285	0.0016*
Statistic for the goodness of fit of the model						
R ²	0.9936		0.9364		0.9725	
adjusted R ²	0.9821		0.8219		0.9231	
RSD	0.3126		1.2589		5.5597	
P	0.7137		0.9424		0.9913	

R²–determination coefficient. adjusted R². RSD–residual standard deviation. P-value of the lack-of-fit test for the model; * - significant coefficients of the model. T–Temperature. P–Pressure. EtOH %–Ethanol as Co-solvent

Supplementary material - Table S2. Regression coefficients and p-Value for Total phenol content and antioxidant activity in their original units and statistics for the fit obtained by multiple linear regression.

Terms of model	Total Phenol Content (mg GAE / g extract)		Antioxidant activity (TEAC) (mmol TE / g extract)	
	Estimate	p-Value	Estimate	p-Value
Constant	4.8792		1.9497	
A: T.	0.7968	0.0196*	-0.0094	0.0406*
B: P	-0.8526	0.2327	-0.1059	0.0129*
C: EtOH %	0.3422	0.1334	0.0092	0.0066*
T × T	-0.0075	0.2824	0.0006	0.1906
T × P	0.0012	0.8871	-0.0013	0.0545
T × EtOH %	0.0079	0.1611	0.0006	0.1048
P × P	0.0093	0.4424	0.0022	0.0242*
P × EtOH %	0.0081	0.2613	0.0007	0.1478
EtOH % × EtOH %	-0.0188	0.0054*	-0.0010	0.0129*
Statistic for the goodness of fit of the model				
R ²	0.9002		0.9437	
adjusted R ²	0.7208		0.8423	
RSD	2.3916		0.5337	
P	0.7128		0.6075	

R²–determination coefficient. adjusted R². RSD–residual standard deviation. P-value of the lack-of-fit test for the model; * - significant coefficients of the model. T–Temperature. P–Pressure. EtOH %–Ethanol as Co-solvent

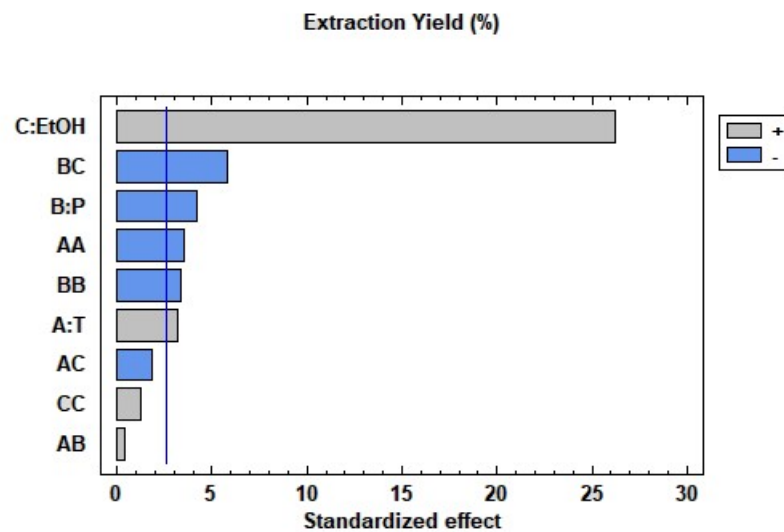


Figure S1. Pareto Chart of the combined effects of temperature (30–70 °C), pressure (25–55 MPa), and ethanol as co-solvent (0–50% v/v) on extraction yield from *Coccomyxa onubensis*. Abbreviation: temperature (T); pressure (P) and ethanol as co-solvent (EtOH).

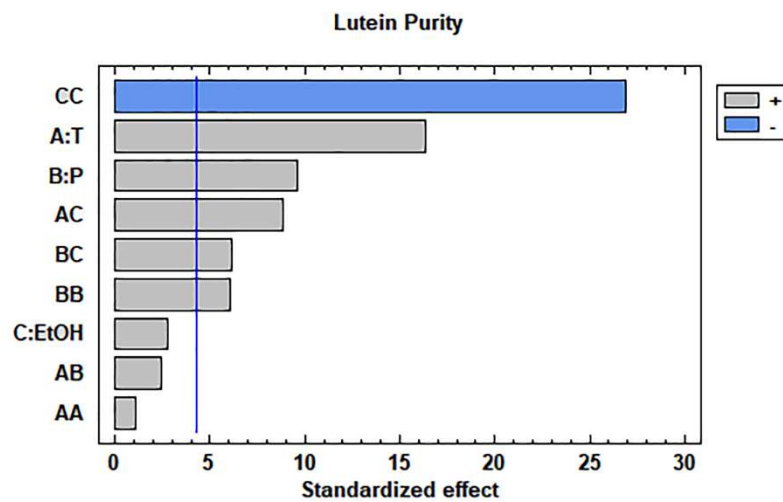


Figure S2. Pareto Chart of the combined effects of temperature (30–70 °C), pressure (25–55 MPa), and ethanol as co-solvent (0%–50% v/v) on lutein purity (LP) from *Coccomyxa onubensis*. Abbreviation: temperature (T), pressure (P), and ethanol as co-solvent (EtOH).

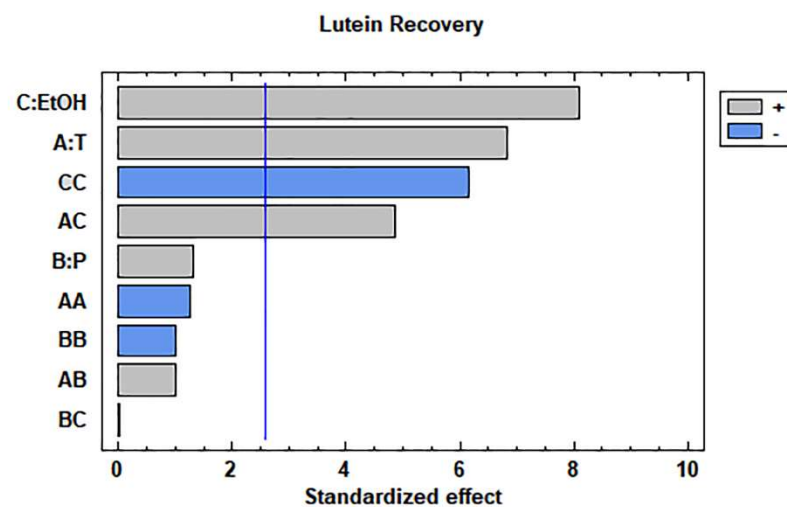


Figure S3. Pareto Chart of the combined effects of temperature (30–70 °C), pressure (25–55 MPa), and ethanol as co-solvent (0–50% v/v) on lutein recovery (LR) from *Coccomyxa onubensis*. Abbreviation: temperature (T), pressure (P), and ethanol as co-solvent (EtOH).

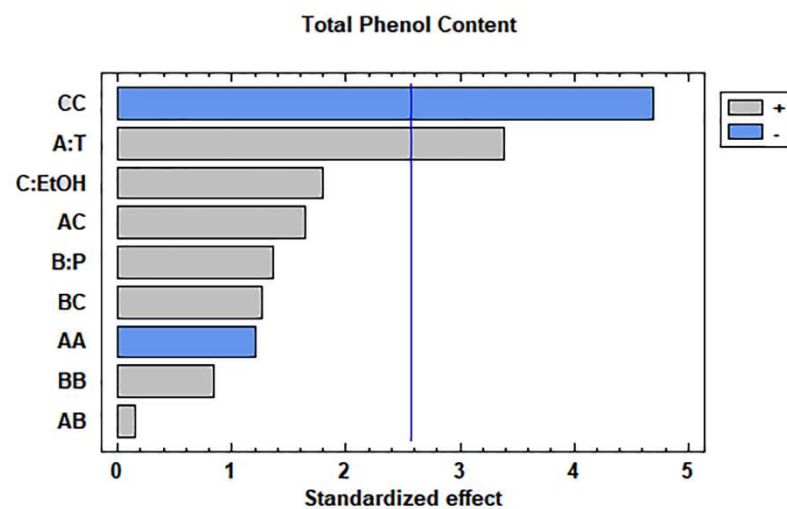


Figure S4. Pareto Chart of the combined effects of temperature (30–70 °C), pressure (25–55 MPa), and ethanol as co-solvent (0–50% v/v) on total phenol content (TPC) from *Coccomyxa onubensis*. Abbreviation: temperature (T), pressure (P), and ethanol as co-solvent (EtOH).

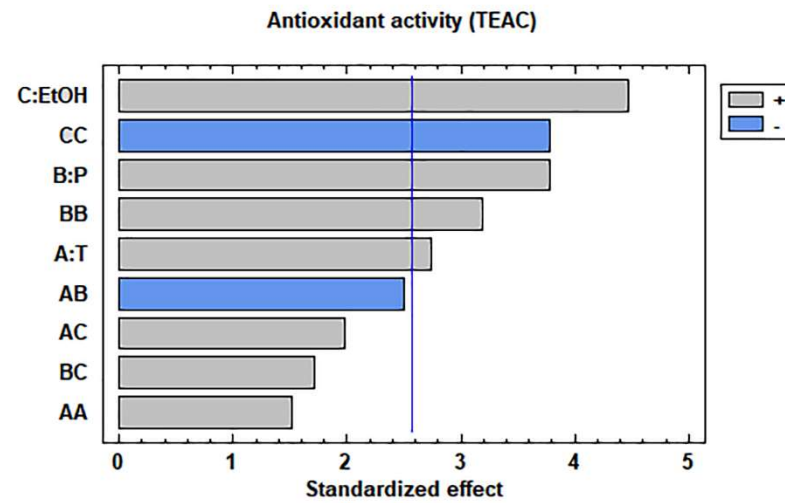


Figure S5. Pareto Chart of the combined effects of temperature (30–70 °C), pressure (25–55 MPa), and ethanol as co-solvent (0–50% v/v) on antioxidant capacity (TEAC) from *Coccomyxa onubensis*. Abbreviation: temperature (T), pressure (P), and ethanol as co-solvent (EtOH).