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Supplementary material

Maximizing the Extraction of Bioactive Compounds from *Diospyros kaki* Peel Through the Use of a Pulsed Electric Field and Ultrasound Extraction

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Section S1. Total Polyphenol Content (TPC) Determination

Following a previously established methodology [1], the total polyphenol content (TPC) of the extracts was determined using the Folin-Ciocalteu assay. In brief, a 1.5 mL Eppendorf tube was filled with 100 μL of persimmon peel extracts and 100 μL of Folin-Ciocalteu reagent. The solution was heated at 40 °C for 20 minutes before 800 μL of Na_2CO_3 solution (5% *w*/*v*) was added. Ultimately, a Shimadzu spectrophotometer (UV-1700, Shimadzu Europa GmbH, Duisburg, Germany) was used to record the absorbance at 740 nm. A calibration curve was further prepared using gallic acid as a standard compound. The TPC (C_{TP}) was expressed as mg gallic acid equivalents (GAE) per L. The extraction yield in total polyphenols (Y_{TP}) was expressed as mg GAE per g of dry weight (dw), using the following Equation (S1):

$$Y_{TP}\ (\text{mg GAE/g dw}) = \frac{C_{TP} \times V}{w} \quad \text{(S1)}$$

where V is the volume of the extraction medium (in L) and w is the dry weight of the sample (in g).

Section S2. Ferric Reducing Antioxidant Power (FRAP) Assay

An earlier described technique was used to assess the FRAP [2]. The sample extracts were combined with 0.05 mL of $FeCl_3$ solution (4 mM in 0.05 M HCl) in an Eppendorf tube, and the mixture was then incubated at 37 °C for 30 min. Following the addition of 0.90 mL of the TPTZ solution (1 mM in 0.05 M HCl), the absorbance at 620 nm was measured after 5 minutes. Ascorbic acid was used to create a calibration curve. Ferric reducing antioxidant power (P_R) was determined as μmol ascorbic acid equivalents (AAE) per g of dw, using the following Equation (S2):

$$P_R\ (\mu\text{mol AAE/g dw}) = \frac{C_{AA} \times V}{w} \quad \text{(S2)}$$

where V is the volume of the extraction medium (in L) and w is the dry weight of the sample (in g).

Section S3. DPPH Radical Scavenging Activity

An approach that has been used before was used to evaluate the DPPH radicals' absorption activity [1]. In brief, 25 μL of the obtained extract were carefully mixed with 975 μL of 100 μM DPPH solution. The solution's absorbance was then measured at 515 nm ($A_{515(i)}$) after mixing and after 30 min of incubation in the absence of light ($A_{515(f)}$). The antiradical activity (A_{AR}) was calculated employing Equation (S3):

$$A_{AR}\ (\mu\text{mol DPPH/g dw}) = \frac{\Delta A}{\varepsilon \times l \times C} \times Y_{TP} \quad \text{(S3)}$$

where $\Delta A = A_{515(i)} - A_{515(f)}$; ε (DPPH) = 11,126 × 10^{-6} μM^{-1} cm^{-1}; $C = C_{TP} \times 0.025$; Y_{TP} is the total polyphenol yield of the extract (mg/g), and l is the path length (1 cm).

Section S4. Hydrogen peroxide (H_2O_2) scavenging assay

A previously mentioned method was applied for the H_2O_2 scavenging assay [3]. 400 µL of the extract along with 600 µL of H_2O_2 solution (40 mM, prepared in phosphate buffer, pH 7.4) was added in an Eppendorf tube. The absorbance at 230 nm was recorded after 10 min. The capacity to scavenge the H_2O_2 was expressed as:

$$\% \text{ Scavenging of } H_2O_2 = \frac{A_o - (A - A_c)}{A_o} \times 100 \quad \text{(S4)}$$

where, A_o, A_c, and A are the absorbance of the blank solution, the extract solution in the absence of hydrogen peroxide and sample, respectively.

Anti-hydrogen peroxide activity (A_{AHP}) was determined as µmol ascorbic acid equivalents (AAE) per g of dw, using an ascorbic acid calibration curve (C_{AA}, 50–500 µmol/L in 0.05 M HCl), using the following equation:

$$A_{AHP} \text{ (µmol AAE/g dw)} = \frac{C_{AA} \times V}{w} \quad \text{(S5)}$$

where, V is the volume of the extraction medium (in L) and w is the dry weight of the sample (in g).

Section S5. Ascorbic Acid (AA) Content

A colorimetric test created by Dani et al. [4] was used to evaluate the ascorbic acid (AA) concentration. 500 µL of 10% (*v*/*v*) Folin-Ciocalteu reagent was added to 900 µL of 10% (*w*/*v*) trichloroacetic acid after 100 µL of sample persimmon peel extract was added. After 10 minutes, the absorbance was measured at 760 nm. Ascorbic acid was used to prepare a standard curve.

Section S6. Determination of Total Carotenoid Content (TCC)

The estimation of carotenoid content was carried out using a previously reported method [5]. The extraction step involved adding 10 mL of ethanol to 1 g of each sample and stirring for 30 min at 300 rpm at room temperature. The mixture was then placed in an ice bath for 5 min with intermittent shaking, followed by centrifugation for 5 min at 3600× *g*. The resulting extract was used to determine the carotenoid content by measuring its absorbance at 450 nm and by using a standard β-carotene calibration curve.

Section S7. HPLC-Based Determination of the Rutin Content and Other Polyphenolic Compounds

An HPLC system was used to evaluate the sample extracts [5]. The study was conducted using Shimadzu CBM-20A liquid chromatography and a Shimadzu SPD-M20A diode array detector (both supplied by Shimadzu Europa GmbH in Duisburg, Germany). The compounds were separated using a Phenomenex Luna C18(2) column from Phenomenex Inc. in Torrance, California, maintained at 40 °C (100 Å, 5 µm, 4.6 mm × 250 mm). The mobile phase consisted of 0.5% aqueous formic acid (A) and a mixture of 0.5% formic acid in acetonitrile/water (6:4) (B). The gradient program used was as follows: 0% B to 40% B, then to 50% B in 10 min, to 70% B in another 10 min and then held constant for 10 min. The flow rate of the mobile phase was 1 mL/min. The retention time and absorbance spectrum were compared to those of pure chemical standards to identify the compounds and then quantified using calibration curves (0–50 µg/mL).

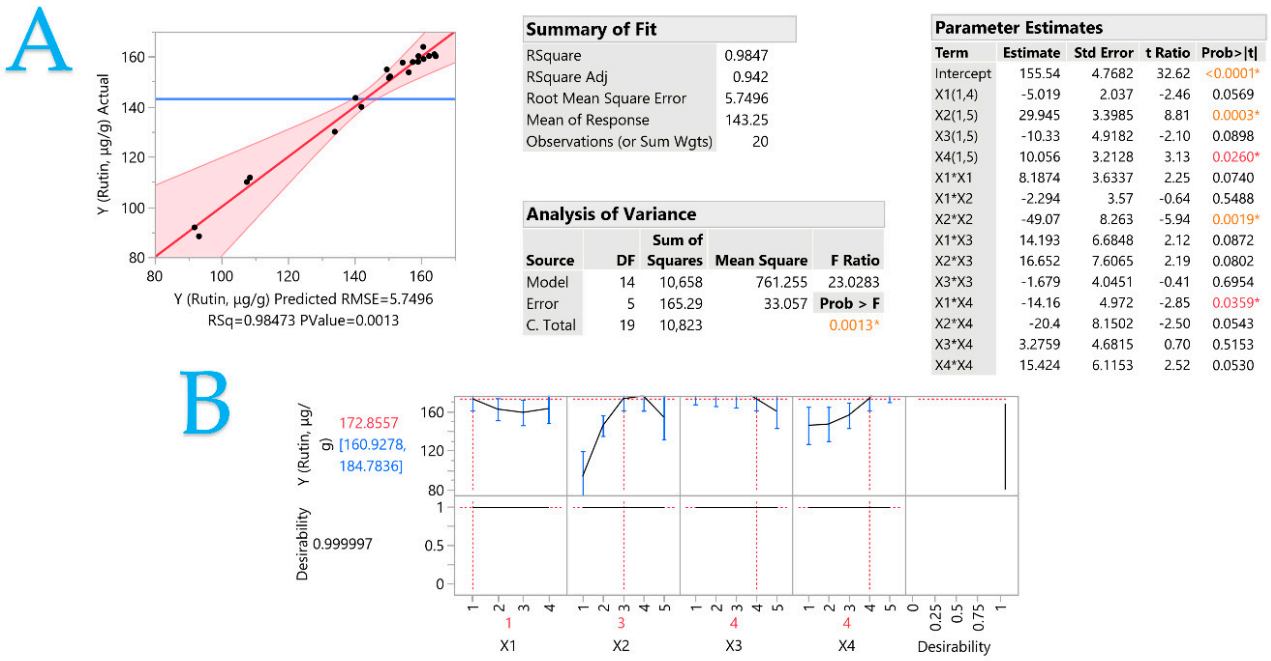


Figure S1. Plots A and B display the actual response versus the predicted response (Rutin, µg/g) for the optimization of Persimmon peels waste extracts carried out with hydroethanolic solutions, different extraction methods, and the desirability function. Asterisks and colored values denote statistically significant values, while inset tables include statistics relevant to the evaluation of the resulting model.

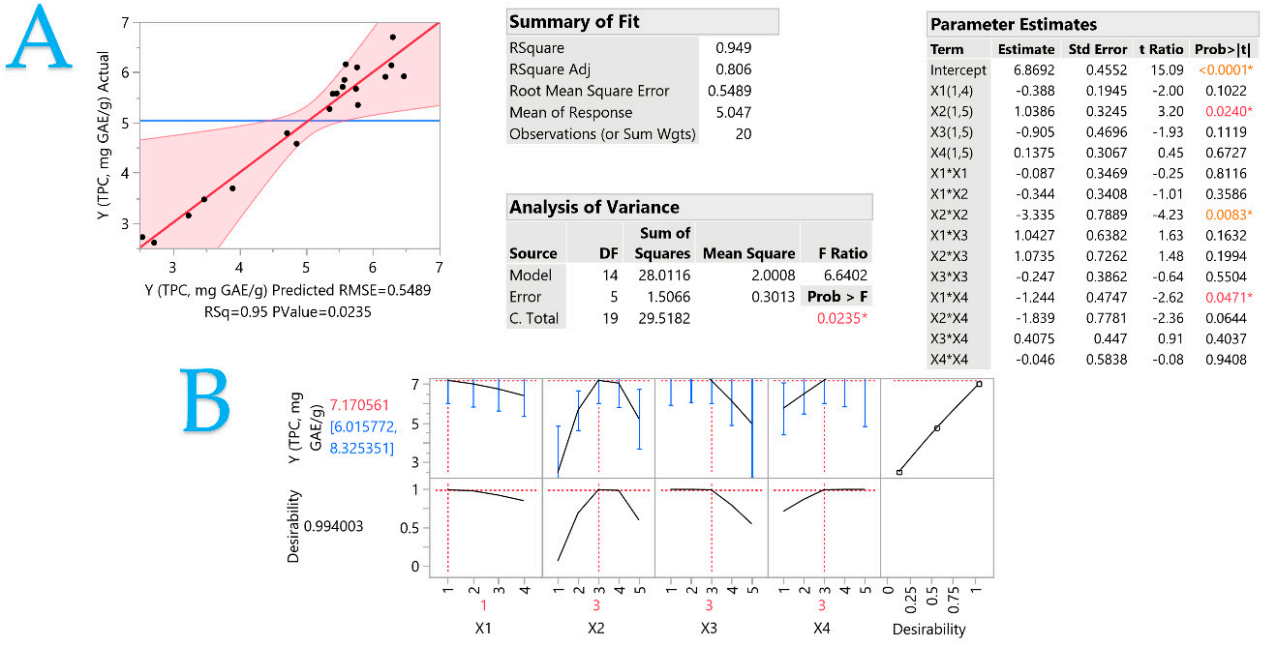
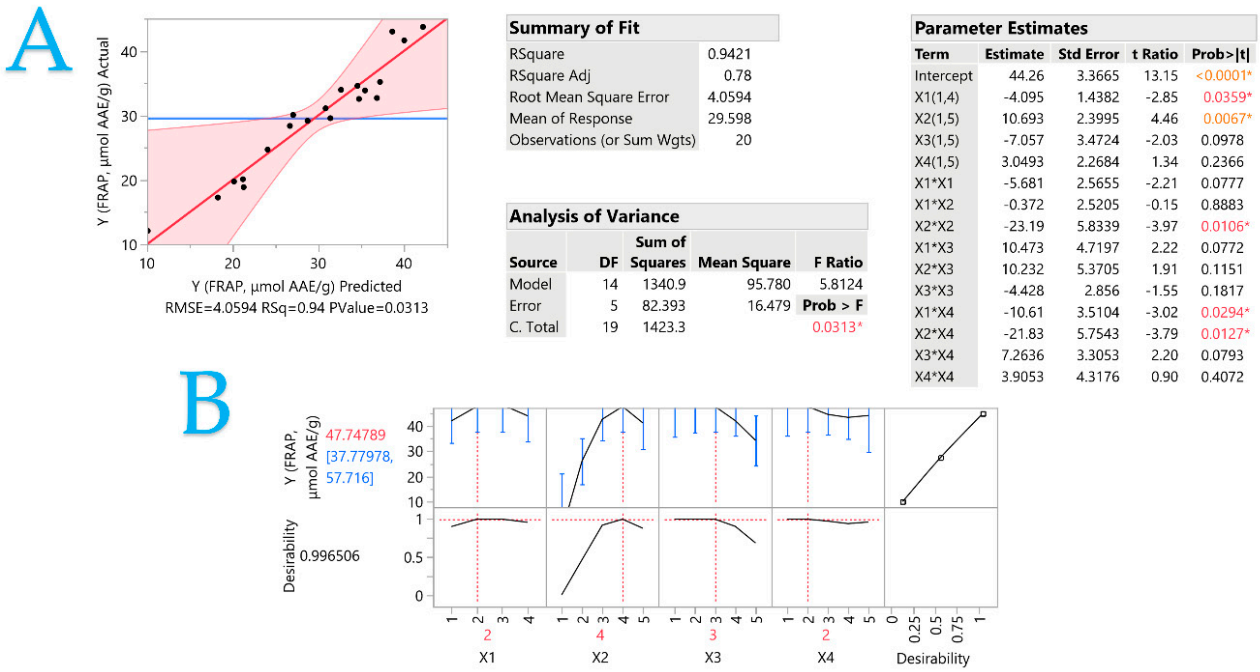


Figure S2. Plots A and B display the actual response versus the predicted response (Total polyphenol content – TPC, mg GAE/g) for the optimization of Persimmon peels waste extracts carried out with hydroethanolic solutions, different extraction methods, and the desirability function. Asterisks and colored values denote statistically significant values, while inset tables include statistics relevant to the evaluation of the resulting model.



Summary of Fit

RSquare	0.9421
RSquare Adj	0.78
Root Mean Square Error	4.0594
Mean of Response	29.598
Observations (or Sum Wgts)	20

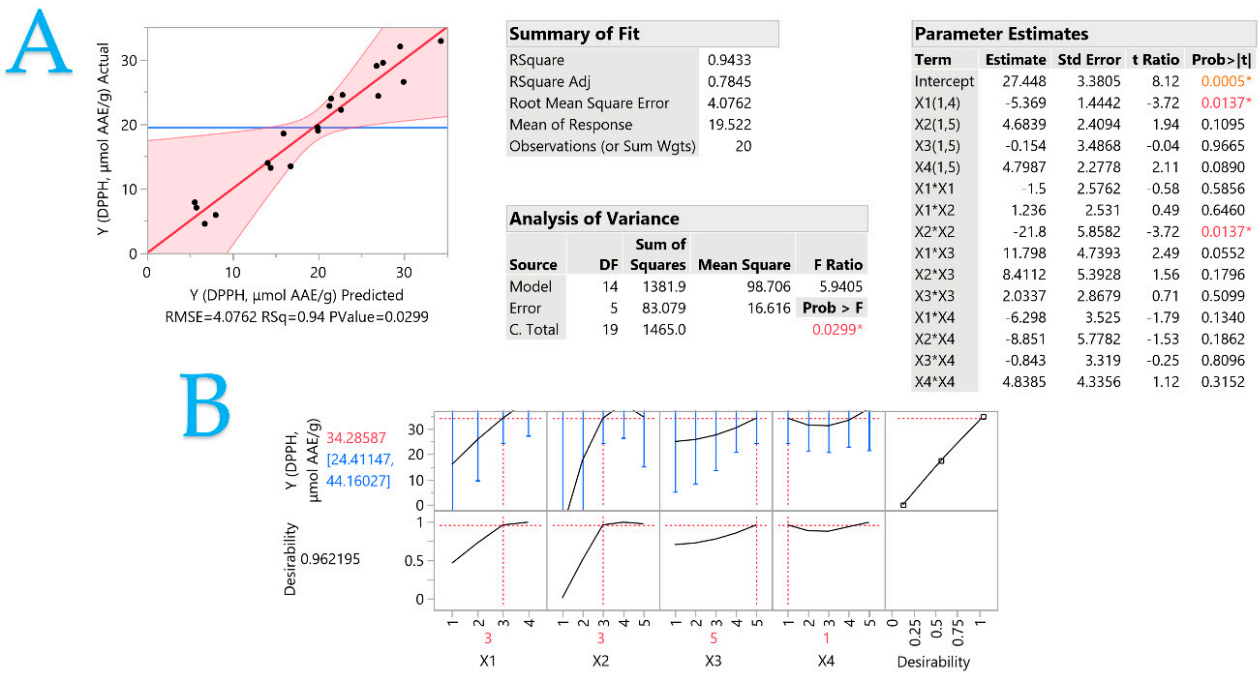
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	14	1340.9	95.780	5.8124
Error	5	82.393	16.479	Prob > F
C. Total	19	1423.3		0.0313*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	44.26	3.3665	13.15	<0.0001*
X1(1,4)	-4.095	1.4382	-2.85	0.0359*
X2(1,5)	10.693	2.3995	4.46	0.0067*
X3(1,5)	-7.057	3.4724	-2.03	0.0978
X4(1,5)	3.0493	2.2684	1.34	0.2366
X1*X1	-5.681	2.5655	-2.21	0.0777
X1*X2	-0.372	2.5205	-0.15	0.8883
X2*X2	-23.19	5.8339	-3.97	0.0106*
X1*X3	10.473	4.7197	2.22	0.0772
X2*X3	10.232	5.3705	1.91	0.1151
X3*X3	-4.428	2.856	-1.55	0.1817
X1*X4	-10.61	3.5104	-3.02	0.0294*
X2*X4	-21.83	5.7543	-3.79	0.0127*
X3*X4	7.2636	3.3053	2.20	0.0793
X4*X4	3.9053	4.3176	0.90	0.4072

Figure S3. Plots A and B display the actual response versus the predicted response (FRAP, µmol AAE/g) for the optimization of Persimmon peels waste extracts carried out with hydroethanolic solutions, different extraction methods, and the desirability function. Asterisks and colored values denote statistically significant values, while inset tables include statistics relevant to the evaluation of the resulting model.



Summary of Fit

RSquare	0.9433
RSquare Adj	0.7845
Root Mean Square Error	4.0762
Mean of Response	19.522
Observations (or Sum Wgts)	20

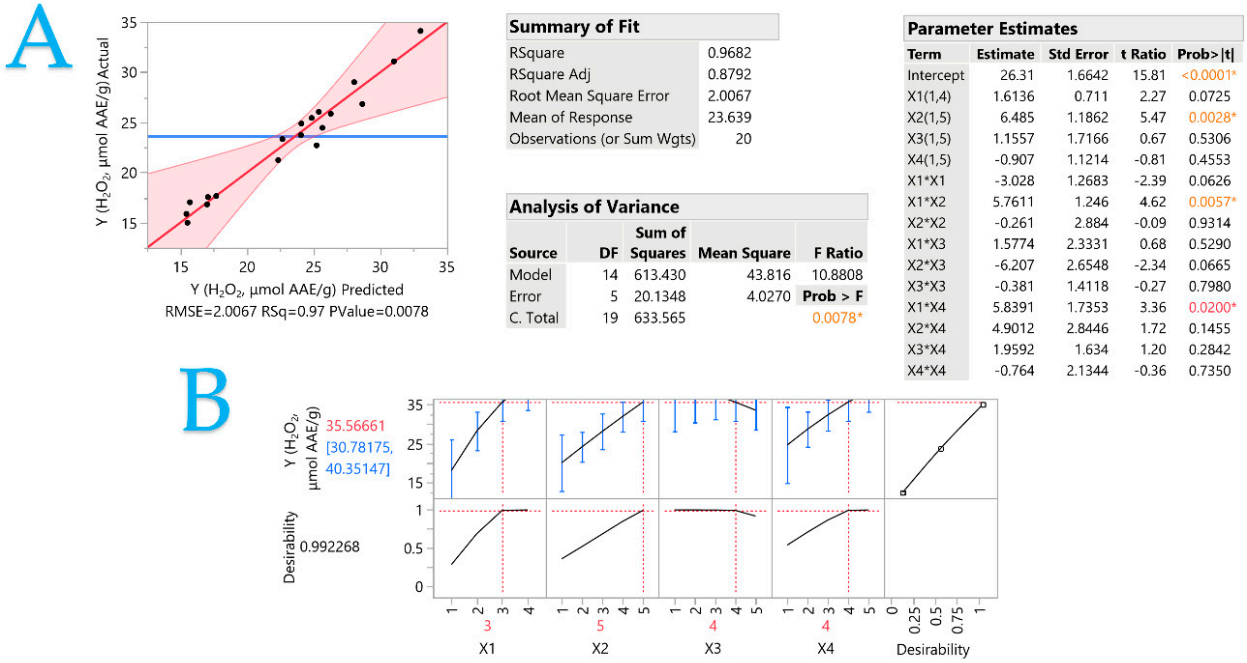
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	14	1381.9	98.706	5.9405
Error	5	83.079	16.616	Prob > F
C. Total	19	1465.0		0.0299*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	27.448	3.3805	8.12	0.0005*
X1(1,4)	-5.369	1.4442	-3.72	0.0137*
X2(1,5)	4.6839	2.4094	1.94	0.1095
X3(1,5)	-0.154	3.4868	-0.04	0.9665
X4(1,5)	4.7987	2.2778	2.11	0.0890
X1*X1	-1.5	2.5762	0.58	0.5856
X1*X2	1.236	2.531	0.49	0.6460
X2*X2	-21.8	5.8582	-3.72	0.0137*
X1*X3	11.798	4.7393	2.49	0.0552
X2*X3	8.4112	5.3928	1.56	0.1796
X3*X3	2.0337	2.8679	0.71	0.5099
X1*X4	-6.298	3.525	-1.79	0.1340
X2*X4	-8.851	5.7782	-1.53	0.1862
X3*X4	-0.843	3.319	-0.25	0.8096
X4*X4	4.8385	4.3356	1.12	0.3152

Figure S4. Plots A and B display the actual response versus the predicted response (DPPH, µmol AAE/g) for the optimization of Persimmon peels waste extracts carried out with hydroethanolic solutions, different extraction methods, and the desirability function. Asterisks and colored values denote statistically significant values, while inset tables include statistics relevant to the evaluation of the resulting model.



Summary of Fit

RSquare	0.9682
RSquare Adj	0.8792
Root Mean Square Error	2.0067
Mean of Response	23.639
Observations (or Sum Wgts)	20

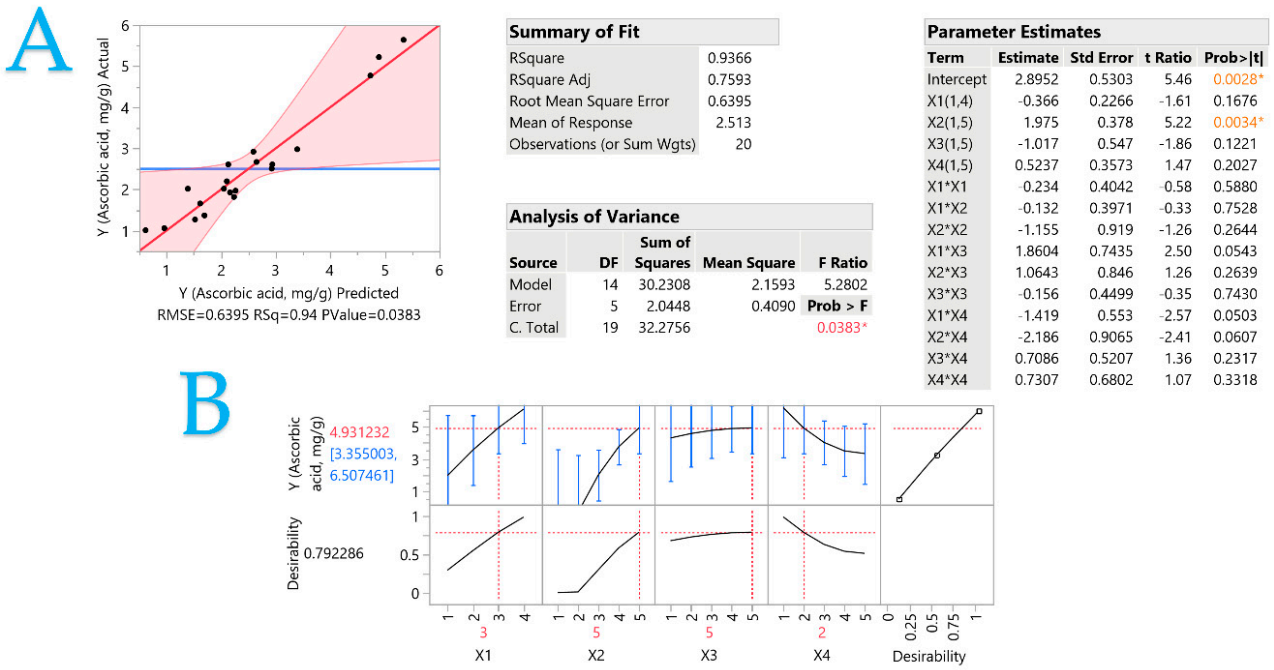
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	14	613.430	43.816	10.8808
Error	5	20.1348	4.0270	Prob > F
C. Total	19	633.565		0.0078*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	26.31	1.6642	15.81	<0.0001*
X1(1,4)	1.6136	0.711	2.27	0.0725
X2(1,5)	6.485	1.1862	5.47	0.0028*
X3(1,5)	1.1557	1.7166	0.67	0.5306
X4(1,5)	-0.907	1.1214	-0.81	0.4553
X1*X1	-3.028	1.2683	-2.39	0.0626
X1*X2	5.7611	1.246	4.62	0.0057*
X2*X2	-0.261	2.884	-0.09	0.9314
X1*X3	1.5774	2.3331	0.68	0.5290
X2*X3	-6.207	2.6548	-2.34	0.0665
X3*X3	-0.381	1.4118	-0.27	0.7980
X1*X4	5.8391	1.7353	3.36	0.0200*
X2*X4	4.9012	2.8446	1.72	0.1455
X3*X4	1.9592	1.634	1.20	0.2842
X4*X4	-0.764	2.1344	-0.36	0.7350

Figure S5. Plots A and B display the actual response versus the predicted response (H_2O_2, µmol AAE/g) for the optimization of Persimmon peels waste extracts carried out with hydroethanolic solutions, different extraction methods, and the desirability function. Asterisks and colored values denote statistically significant values, while inset tables include statistics relevant to the evaluation of the resulting model.



Summary of Fit

RSquare	0.9366
RSquare Adj	0.7593
Root Mean Square Error	0.6395
Mean of Response	2.513
Observations (or Sum Wgts)	20

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	14	30.2308	2.1593	5.2802
Error	5	2.0448	0.4090	Prob > F
C. Total	19	32.2756		0.0383*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	2.8952	0.5303	5.46	0.0028*
X1(1,4)	-0.366	0.2266	-1.61	0.1676
X2(1,5)	1.975	0.378	5.22	0.0034*
X3(1,5)	-1.017	0.547	-1.86	0.1221
X4(1,5)	0.5237	0.3573	1.47	0.2027
X1*X1	-0.234	0.4042	-0.58	0.5880
X1*X2	-0.132	0.3971	-0.33	0.7528
X2*X2	-1.155	0.919	-1.26	0.2644
X1*X3	1.8604	0.7435	2.50	0.0543
X2*X3	1.0643	0.846	1.26	0.2639
X3*X3	-0.156	0.4499	-0.35	0.7430
X1*X4	-1.419	0.553	-2.57	0.0503
X2*X4	-2.186	0.9065	-2.41	0.0607
X3*X4	0.7086	0.5207	1.36	0.2317
X4*X4	0.7307	0.6802	1.07	0.3318

Figure S6. Plots A and B display the actual response versus the predicted response (Ascorbic acid, mg/g) for the optimization of Persimmon peels waste extracts carried out with hydroethanolic solutions, different extraction methods, and the desirability function. Asterisks and colored values denote statistically significant values, while inset tables include statistics relevant to the evaluation of the resulting model.

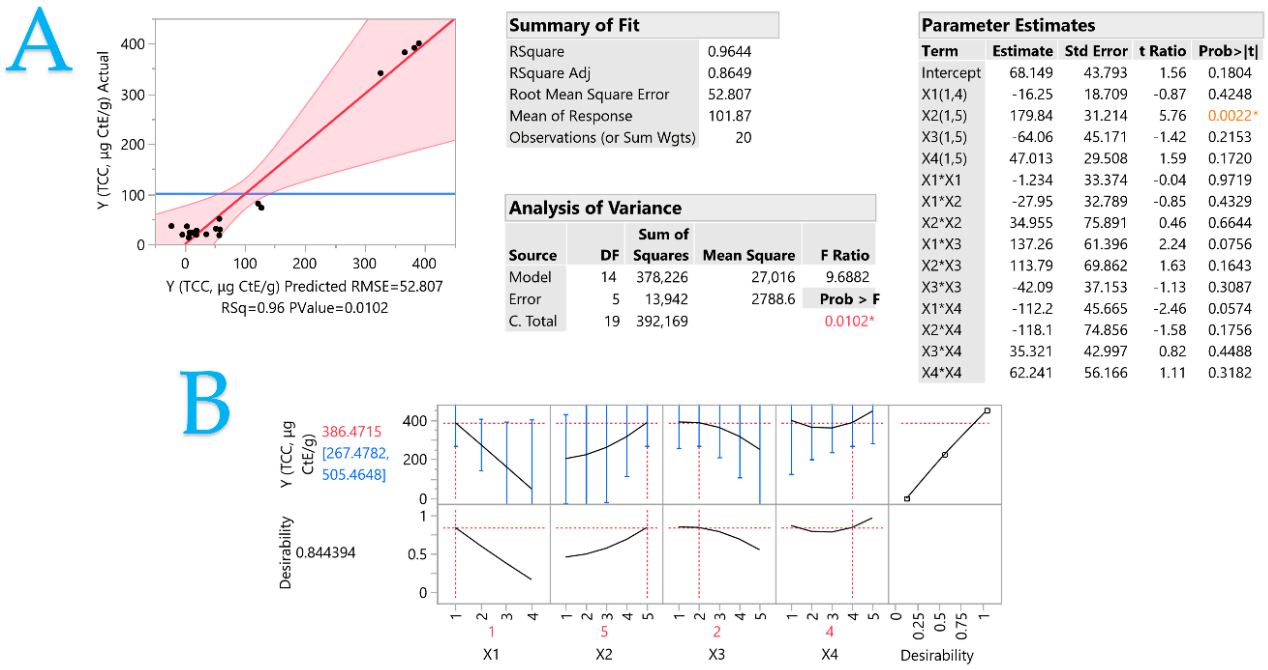


Figure S7. Plots A and B display the actual response versus the predicted response (Total carotenoid content – TCC, µg CtE/g) for the optimization of Persimmon peels waste extracts carried out with hydroethanolic solutions, different extraction methods, and the desirability function. Asterisks and colored values denote statistically significant values, while inset tables include statistics relevant to the evaluation of the resulting model.

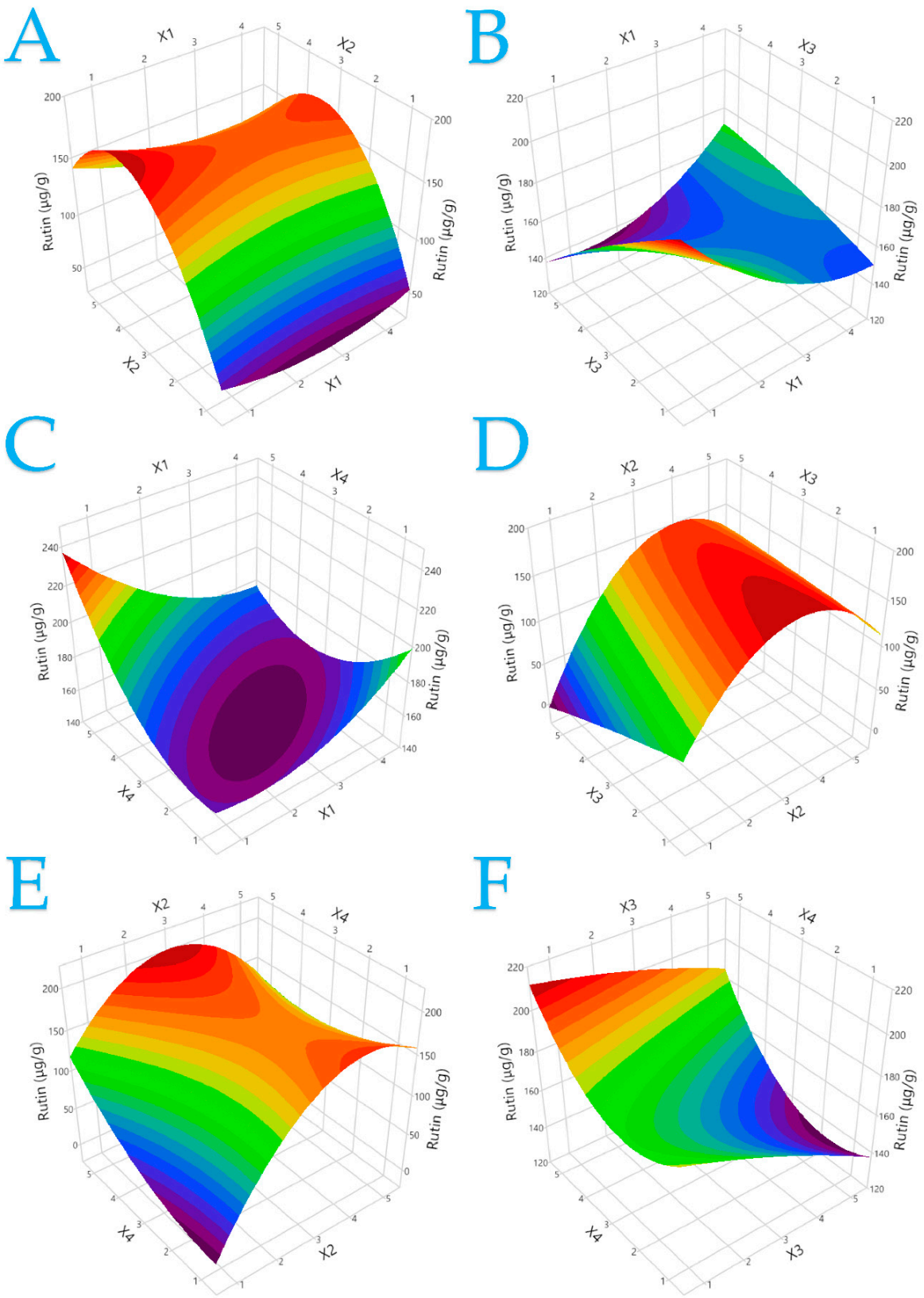


Figure S8. The optimal extraction of Persimmon peels waste extracts using different extraction methods and hydroethanolic solutions is shown in 3D graphs that show the impact of the process variables considered in the response (Rutin, µg/g). Plot (A), covariation of X_1 and X_2; plot (B), covariation of X_1 and X_3; plot (C), covariation of X_1 and X_4; plot (D), covariation of X_2 and X_3; plot (E), covariation of X_2 and X_4; plot (F), covariation of X_3 and X_4.

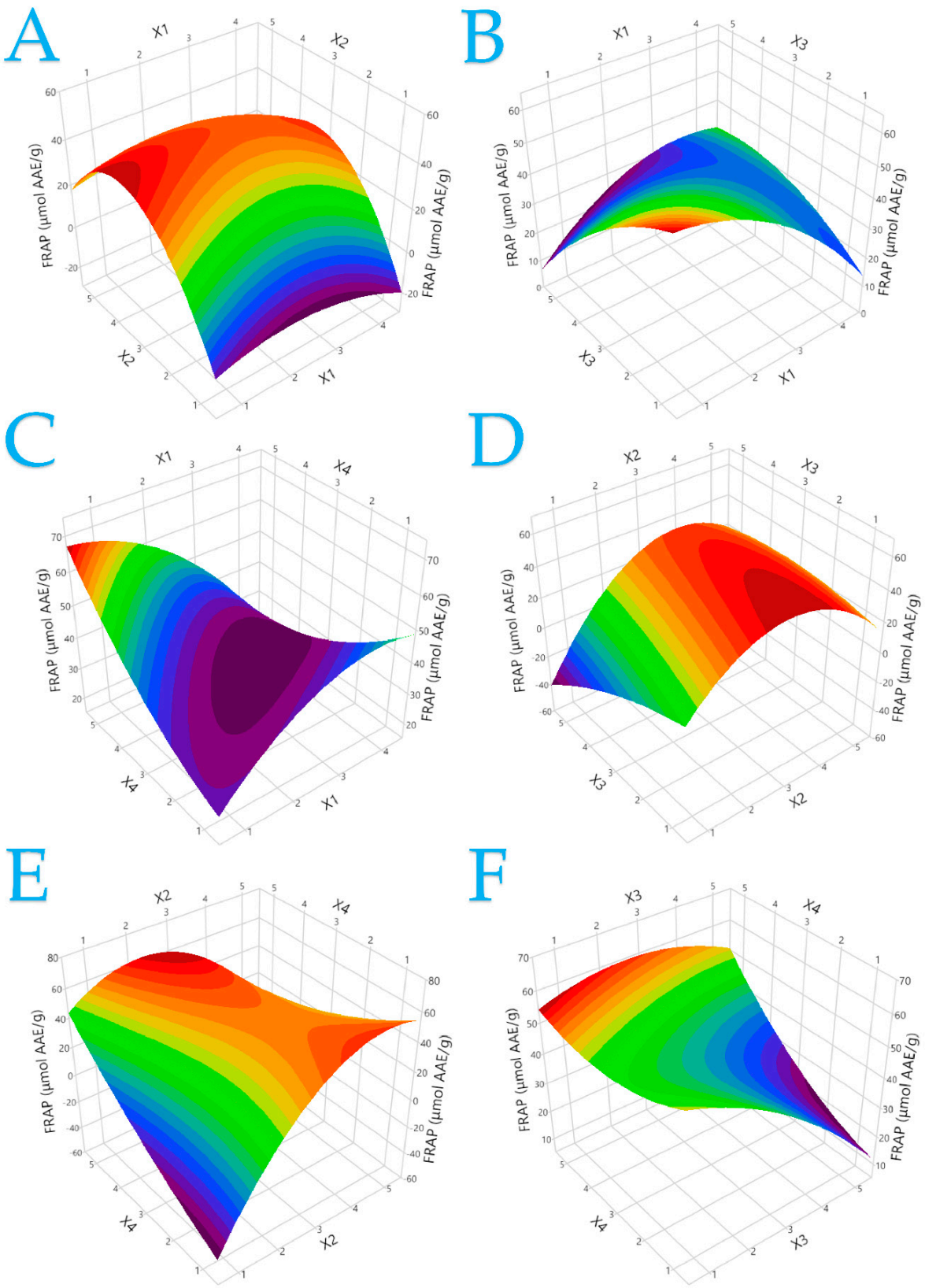


Figure S9. The optimal extraction of Persimmon peels waste extracts using different extraction methods and hydroethanolic solutions is shown in 3D graphs that show the impact of the process variables considered in the response (FRAP, µmol AAE/g). Plot (A), covariation of X_1 and X_2; plot (B), covariation of X_1 and X_3; plot (C), covariation of X_1 and X_4; plot (D), covariation of X_2 and X_3; plot (E), covariation of X_2 and X_4; plot (F), covariation of X_3 and X_4.

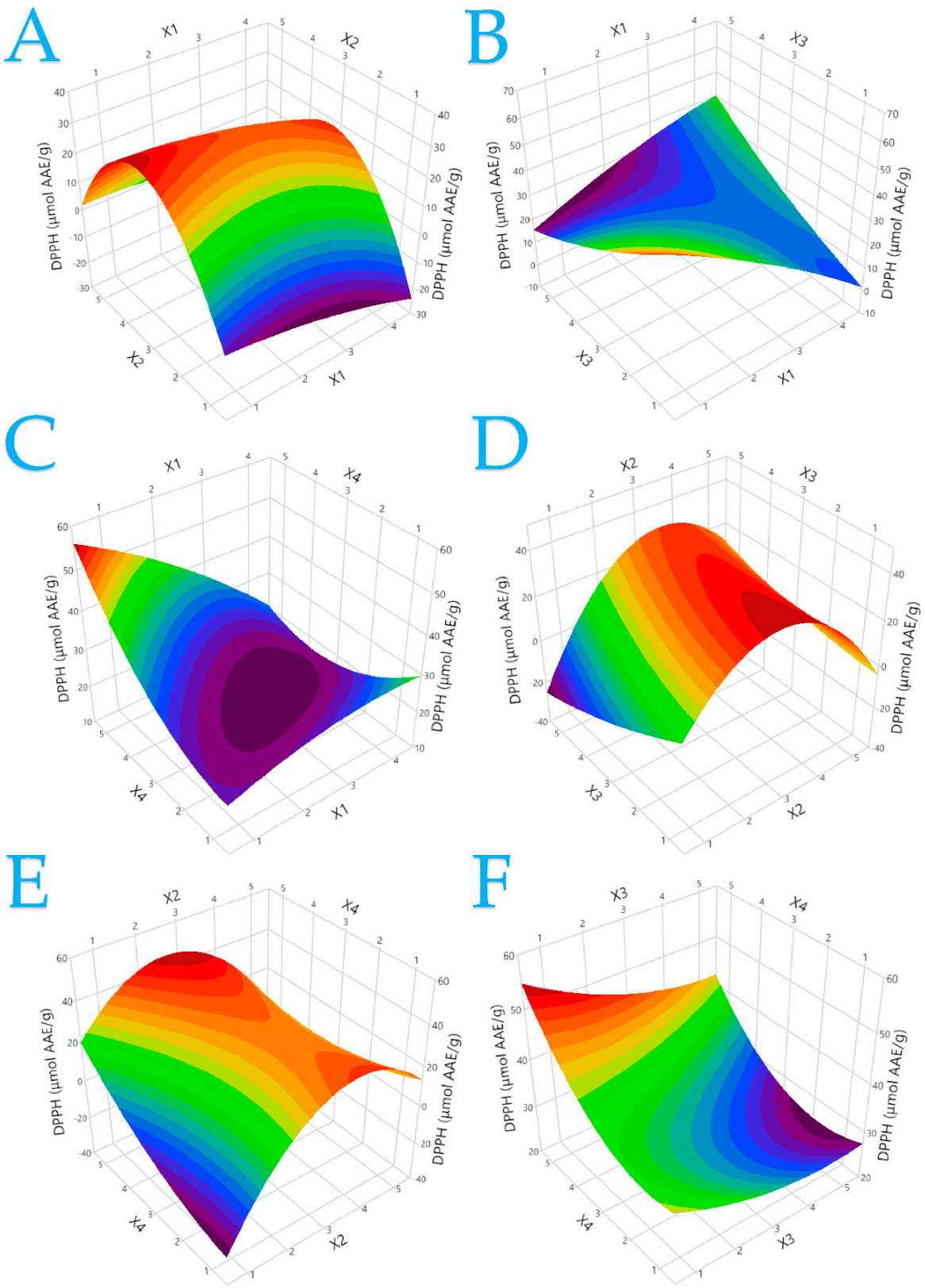


Figure S10. The optimal extraction of Persimmon peels waste extracts using different extraction methods and hydroethanolic solutions is shown in 3D graphs that show the impact of the process variables considered in the response (DPPH, µmol AAE/g). Plot (A), covariation of X_1 and X_2; plot (B), covariation of X_1 and X_3; plot (C), covariation of X_1 and X_4; plot (D), covariation of X_2 and X_3; plot (E), covariation of X_2 and X_4; plot (F), covariation of X_3 and X_4.

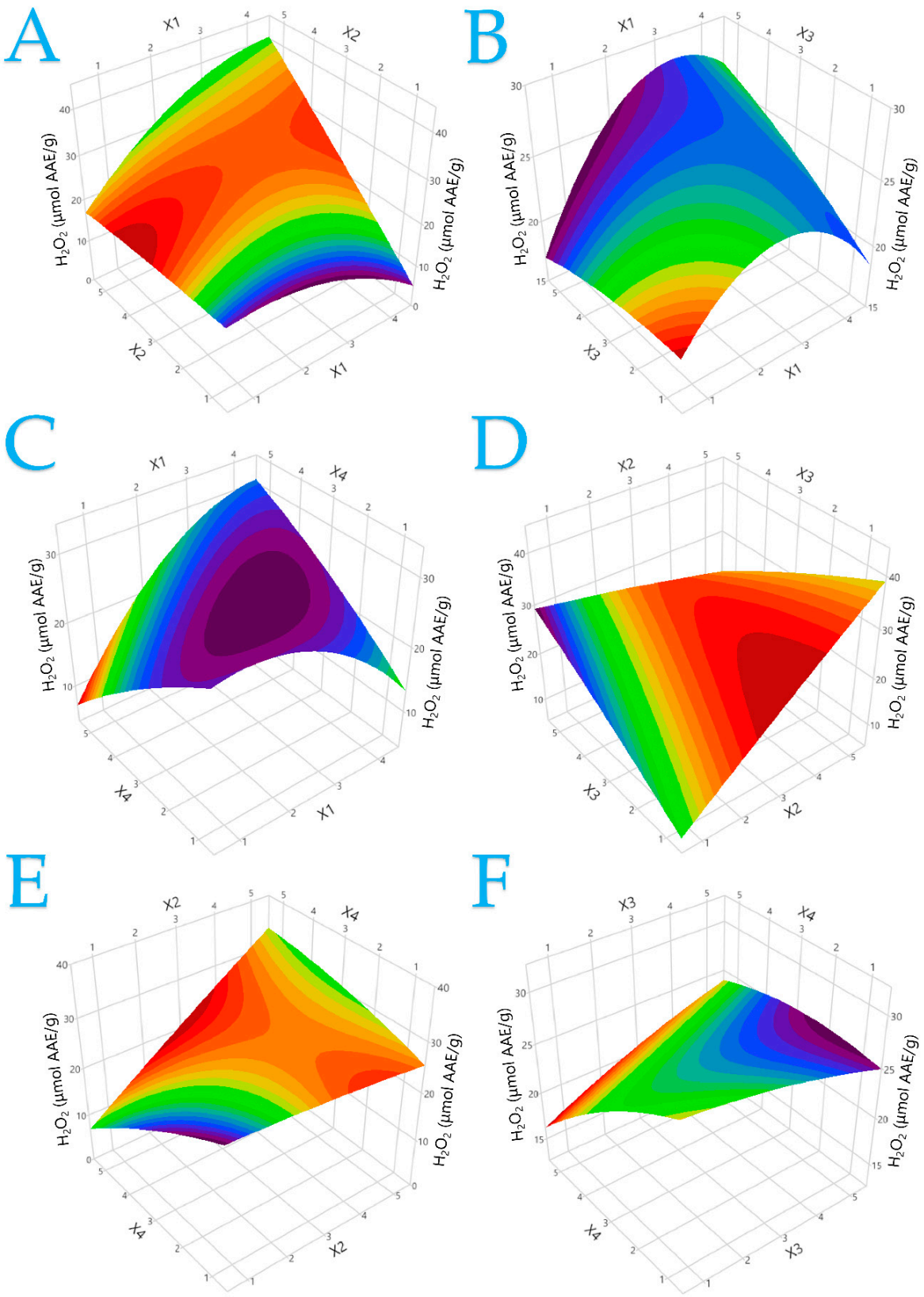


Figure S11. The optimal extraction of Persimmon peels waste extracts using different extraction methods and hydroethanolic solutions is shown in 3D graphs that show the impact of the process variables considered in the response (H_2O_2, µmol AAE/g). Plot (A), covariation of X_1 and X_2; plot (B), covariation of X_1 and X_3; plot (C), covariation of X_1 and X_4; plot (D), covariation of X_2 and X_3; plot (E), covariation of X_2 and X_4; plot (F), covariation of X_3 and X_4.

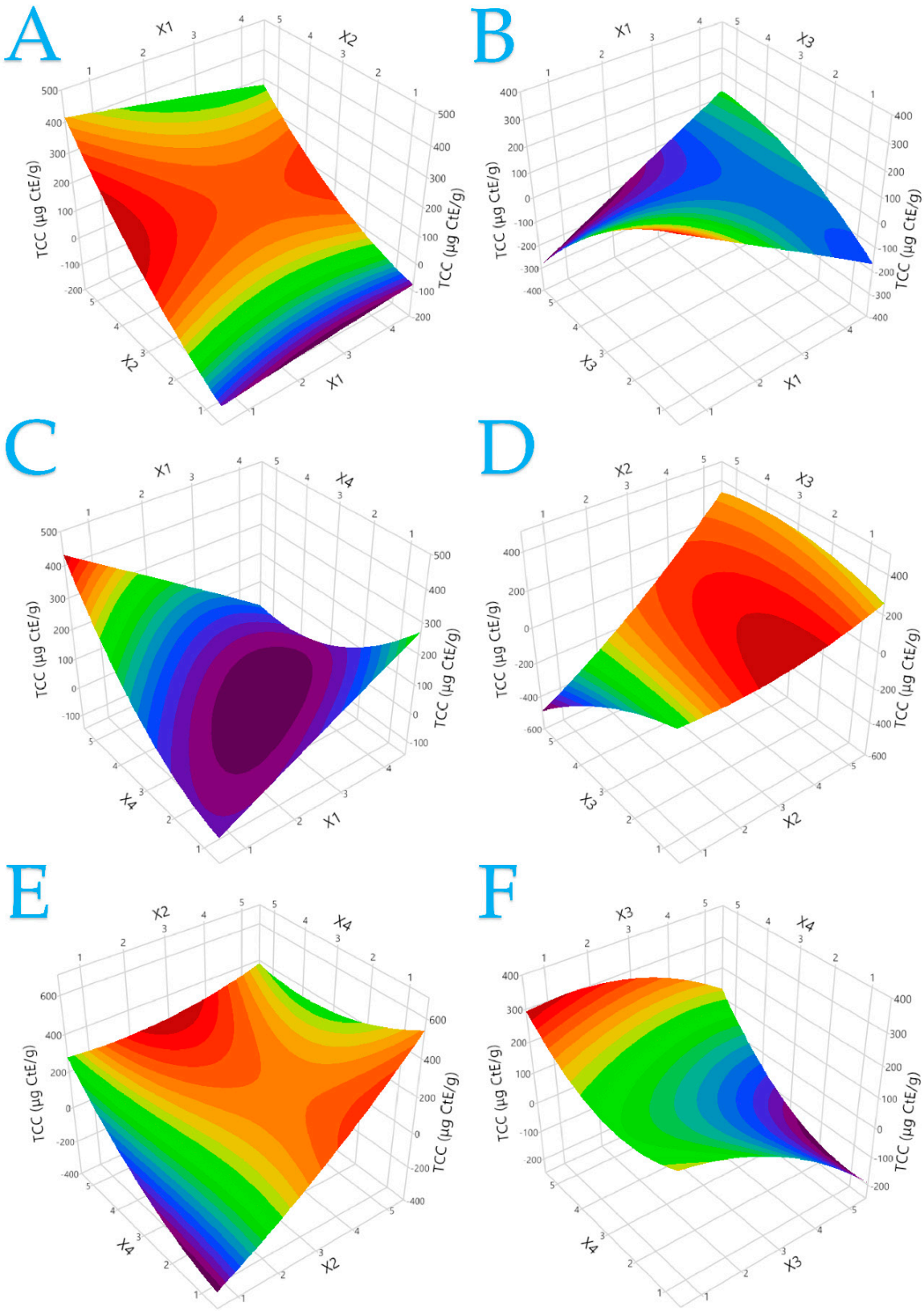


Figure S12. The optimal extraction of Persimmon peels waste extracts using different extraction methods and hydroethanolic solutions is shown in 3D graphs that show the impact of the process variables considered in the response (Total carotenoid content – TCC, µg CtE/g). Plot (A), covariation of X_1 and X_2; plot (B), covariation of X_1 and X_3; plot (C), covariation of X_1 and X_4; plot (D), covariation of X_2 and X_3; plot (E), covariation of X_2 and X_4; plot (F), covariation of X_3 and X_4.

References

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