Supplementary material

Optimization of Extraction Parameters to Enhance the Antioxidant Properties of Pyrus spinosa Fruit extract

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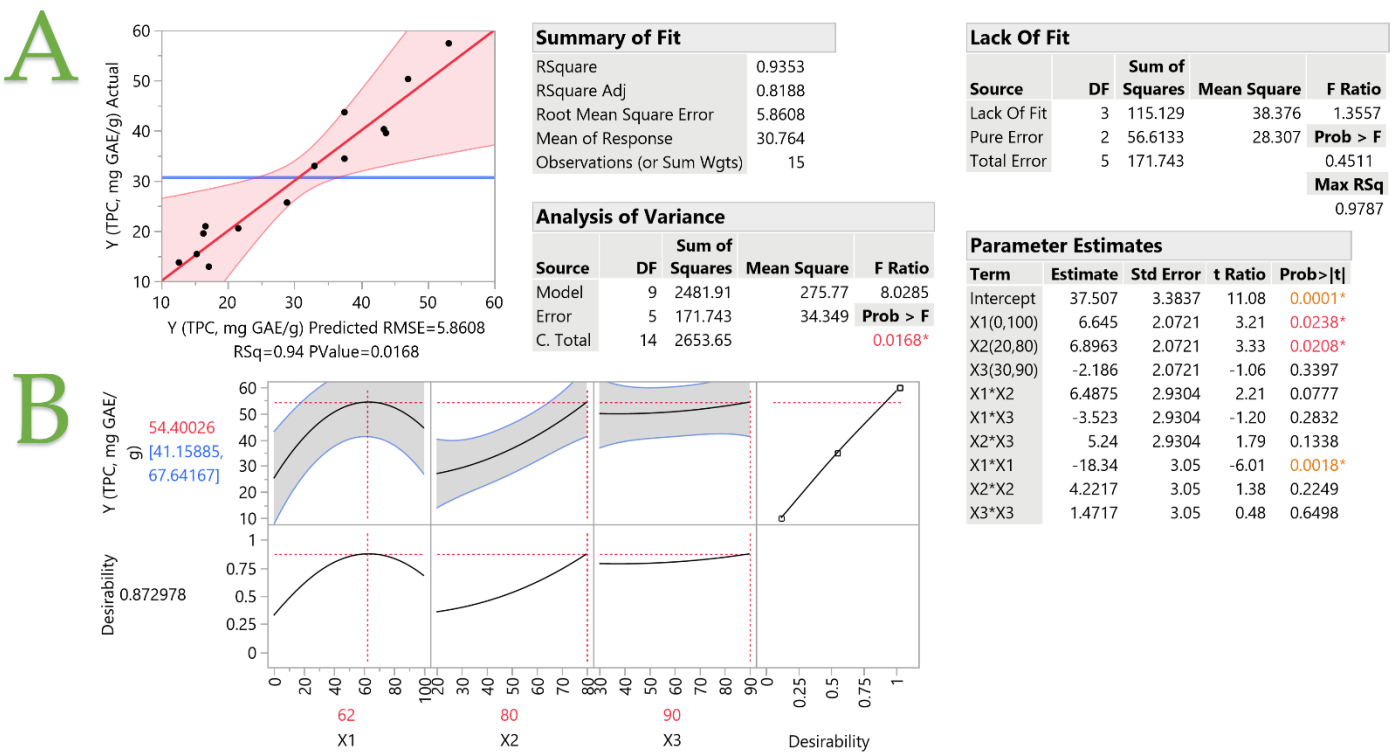
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2.4. Response Surface Methodology (RSM) Optimization of Extraction and Experiment Design

Utilizing the Response Surface Methodology (RSM) technique, the extraction of total polyphenol content (TPC), total flavonoid content (TFC), ascorbic acid content (AAC), polyphenolic compounds, and antioxidant activity was measured using the FRAP, DPPH method aimed for optimal yield. The design's objective was to enhance the content of PS fruit in TPC, TFC, AAC, polyphenolic compounds, and antioxidant activity. This was achieved through adjustments to the extraction procedure involving parameters such as solvent concentration (ethanol, EtOH) represented as C, % v/v, extraction duration denoted as t, min, and extraction temperature indicated as T, °C. An experiment employing a Box-Behnken design with 15 design points formed the basis for optimization, with 3 central points. Process variables were set at three levels, as outlined in Table 1, indicating both coded and actual levels. Analysis of variance (ANOVA) and summary-of-fit tests were employed to establish overall model significance (R^2, p-value) and the significance of model coefficients (equations). Additionally, a second-order polynomial model (Equation S1) was utilized to forecast the dependent variable based on the analyzed independent factors:

$$Y_k = \beta_0 + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \quad \text{(S1)}$$

where X_i and X_j represent the independent variables, and Y_k defines the predicted response variable. The model linear, quadratic, and interaction terms are represented by the intercept and regression coefficients, β_0, β_i, β_{ii}, and β_{ij}, respectively.



Summary of Fit

RSquare	0.9353
RSquare Adj	0.8188
Root Mean Square Error	5.8608
Mean of Response	30.764
Observations (or Sum Wgts)	15

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	2481.91	275.77	8.0285
Error	5	171.743	34.349	Prob > F
C. Total	14	2653.65		0.0168*

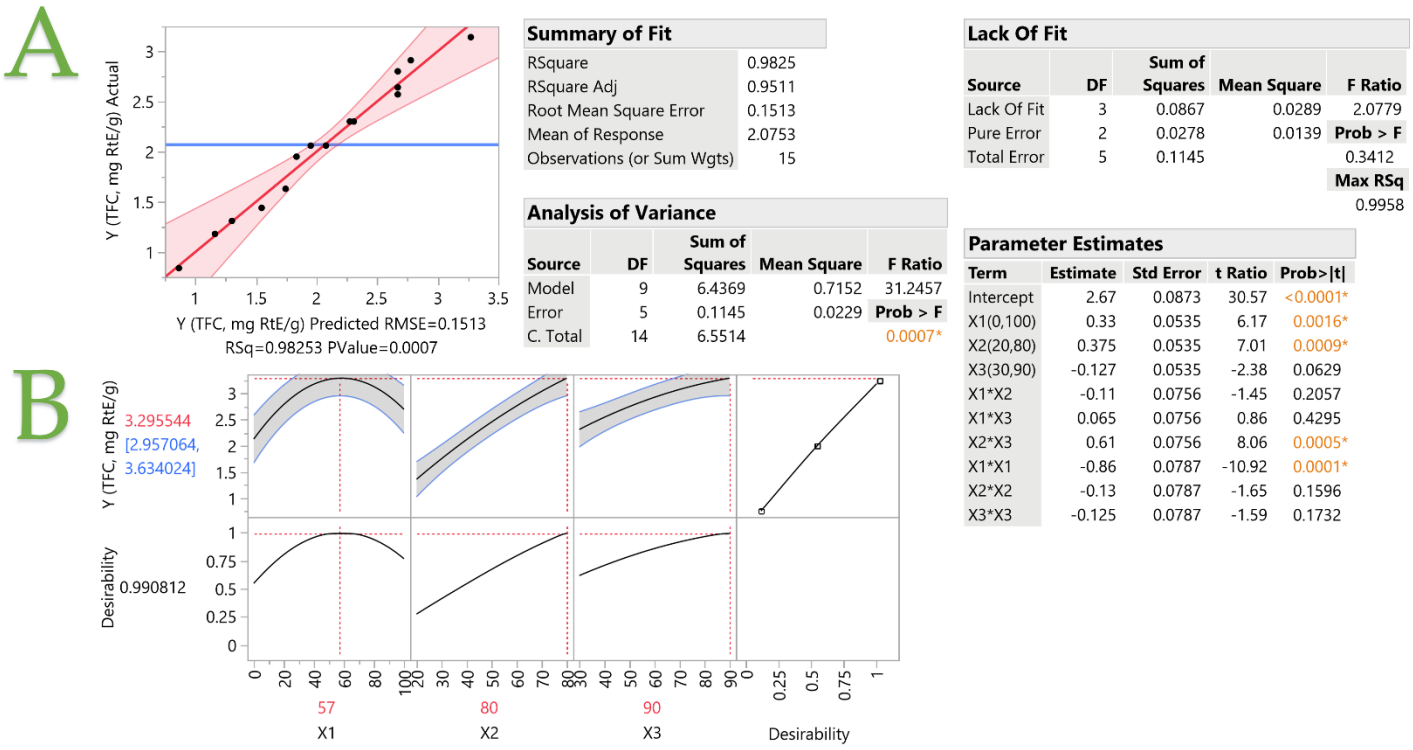
Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	3	115.129	38.376	1.3557
Pure Error	2	56.6133	28.307	Prob > F
Total Error	5	171.743		0.4511
				Max RSq
				0.9787

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	37.507	3.3837	11.08	0.0001*
X1(0,100)	6.645	2.0721	3.21	0.0238*
X2(20,80)	6.8963	2.0721	3.33	0.0208*
X3(30,90)	-2.186	2.0721	-1.06	0.3397
X1*X2	6.4875	2.9304	2.21	0.0777
X1*X3	-3.523	2.9304	-1.20	0.2832
X2*X3	5.24	2.9304	1.79	0.1338
X1*X1	-18.34	3.05	-6.01	0.0018*
X2*X2	4.2217	3.05	1.38	0.2249
X3*X3	1.4717	3.05	0.48	0.6498

Figure S1. Plot **A** displays the actual response versus the predicted response (Total polyphenol content – TPC, mg GAE/g) for the optimization of PS fruit extracts using hydroethanolic solutions, and different extraction parameters, and plot **B** displays 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	0.9825
RSquare Adj	0.9511
Root Mean Square Error	0.1513
Mean of Response	2.0753
Observations (or Sum Wgts)	15

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	6.4369	0.7152	31.2457
Error	5	0.1145	0.0229	Prob > F
C. Total	14	6.5514		0.0007*

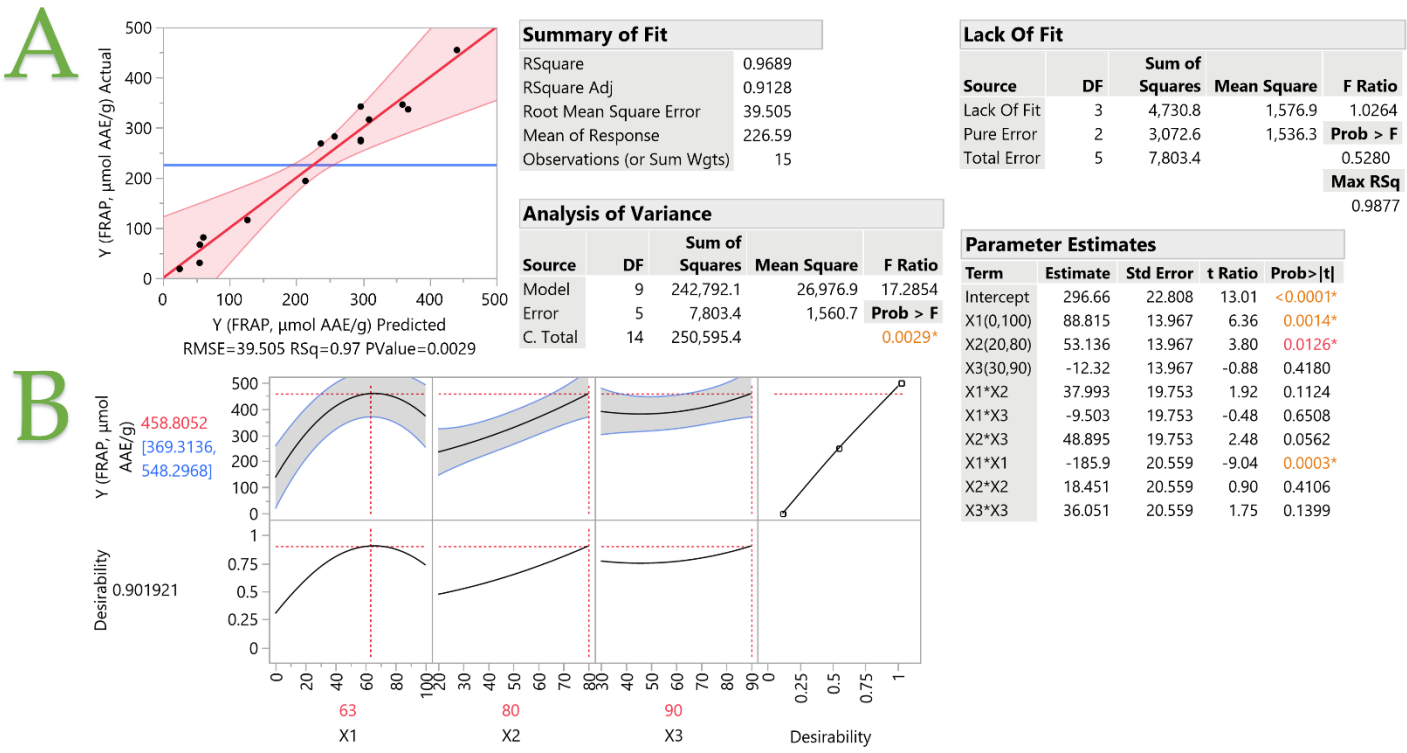
Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	3	0.0867	0.0289	2.0779
Pure Error	2	0.0278	0.0139	Prob > F
Total Error	5	0.1145		0.3412
				Max RSq
				0.9958

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	2.67	0.0873	30.57	<0.0001*
X1(0,100)	0.33	0.0535	6.17	0.0016*
X2(20,80)	0.375	0.0535	7.01	0.0009*
X3(30,90)	-0.127	0.0535	-2.38	0.0629
X1*X2	-0.11	0.0756	-1.45	0.2057
X1*X3	0.065	0.0756	0.86	0.4295
X2*X3	0.61	0.0756	8.06	0.0005*
X1*X1	-0.86	0.0787	-10.92	0.0001*
X2*X2	-0.13	0.0787	-1.65	0.1596
X3*X3	-0.125	0.0787	-1.59	0.1732

Figure S2. Plot **A** displays the actual response versus the predicted response (Total flavonoid content – TFC, mg RtE/g) for the optimization of PS fruit extracts using hydroethanolic solutions, and different extraction parameters, and plot **B** displays the desirability function. Asterisks and colored values denote statistically significant values, while inset tables include statistics relevant to the evaluation of the resulting model.



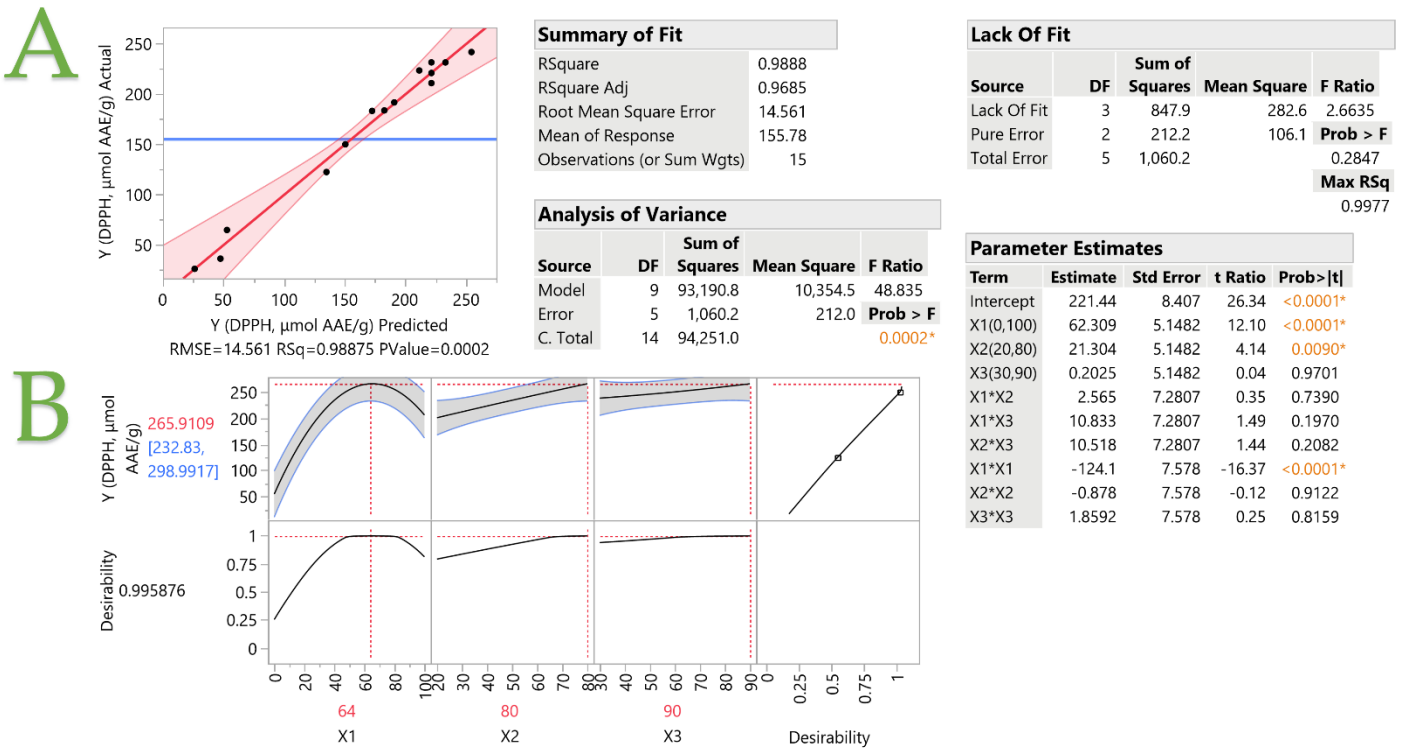
Summary of Fit	
RSquare	0.9689
RSquare Adj	0.9128
Root Mean Square Error	39.505
Mean of Response	226.59
Observations (or Sum Wgts)	15

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	242,792.1	26,976.9	17.2854
Error	5	7,803.4	1,560.7	Prob > F
C. Total	14	250,595.4		0.0029*

Lack Of Fit				
Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	3	4,730.8	1,576.9	1.0264
Pure Error	2	3,072.6	1,536.3	Prob > F
Total Error	5	7,803.4		0.5280
				Max RSq
				0.9877

Parameter Estimates				
Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	296.66	22.808	13.01	<0.0001*
X1(0,100)	88.815	13.967	6.36	0.0014*
X2(20,80)	53.136	13.967	3.80	0.0126*
X3(30,90)	-12.32	13.967	-0.88	0.4180
X1*X2	37.993	19.753	1.92	0.1124
X1*X3	-9.503	19.753	-0.48	0.6508
X2*X3	48.895	19.753	2.48	0.0562
X1*X1	-185.9	20.559	-9.04	0.0003*
X2*X2	18.451	20.559	0.90	0.4106
X3*X3	36.051	20.559	1.75	0.1399

Figure S3. Plot **A** displays the actual response versus the predicted response (FRAP, µmol AAE/g) for the optimization of PS fruit extracts using hydroethanolic solutions, and different extraction parameters, and plot **B** displays 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	0.9888
RSquare Adj	0.9685
Root Mean Square Error	14.561
Mean of Response	155.78
Observations (or Sum Wgts)	15

Analysis of Variance				
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	93,190.8	10,354.5	48.835
Error	5	1,060.2	212.0	Prob > F
C. Total	14	94,251.0		0.0002*

Lack Of Fit				
Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	3	847.9	282.6	2.6635
Pure Error	2	212.2	106.1	Prob > F
Total Error	5	1,060.2		0.2847
				Max RSq
				0.9977

Parameter Estimates				
Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	221.44	8.407	26.34	<0.0001*
X1(0,100)	62.309	5.1482	12.10	<0.0001*
X2(20,80)	21.304	5.1482	4.14	0.0090*
X3(30,90)	0.2025	5.1482	0.04	0.9701
X1*X2	2.565	7.2807	0.35	0.7390
X1*X3	10.833	7.2807	1.49	0.1970
X2*X3	10.518	7.2807	1.44	0.2082
X1*X1	-124.1	7.578	-16.37	<0.0001*
X2*X2	-0.878	7.578	-0.12	0.9122
X3*X3	1.8592	7.578	0.25	0.8159

Figure S4. Plot **A** displays the actual response versus the predicted response (DPPH, µmol AAE/g) for the optimization of PS fruit extracts using hydroethanolic solutions, and different extraction parameters, and plot **B** displays the desirability function. Asterisks and colored values denote statistically significant values, while inset tables include statistics relevant to the evaluation of the resulting model.

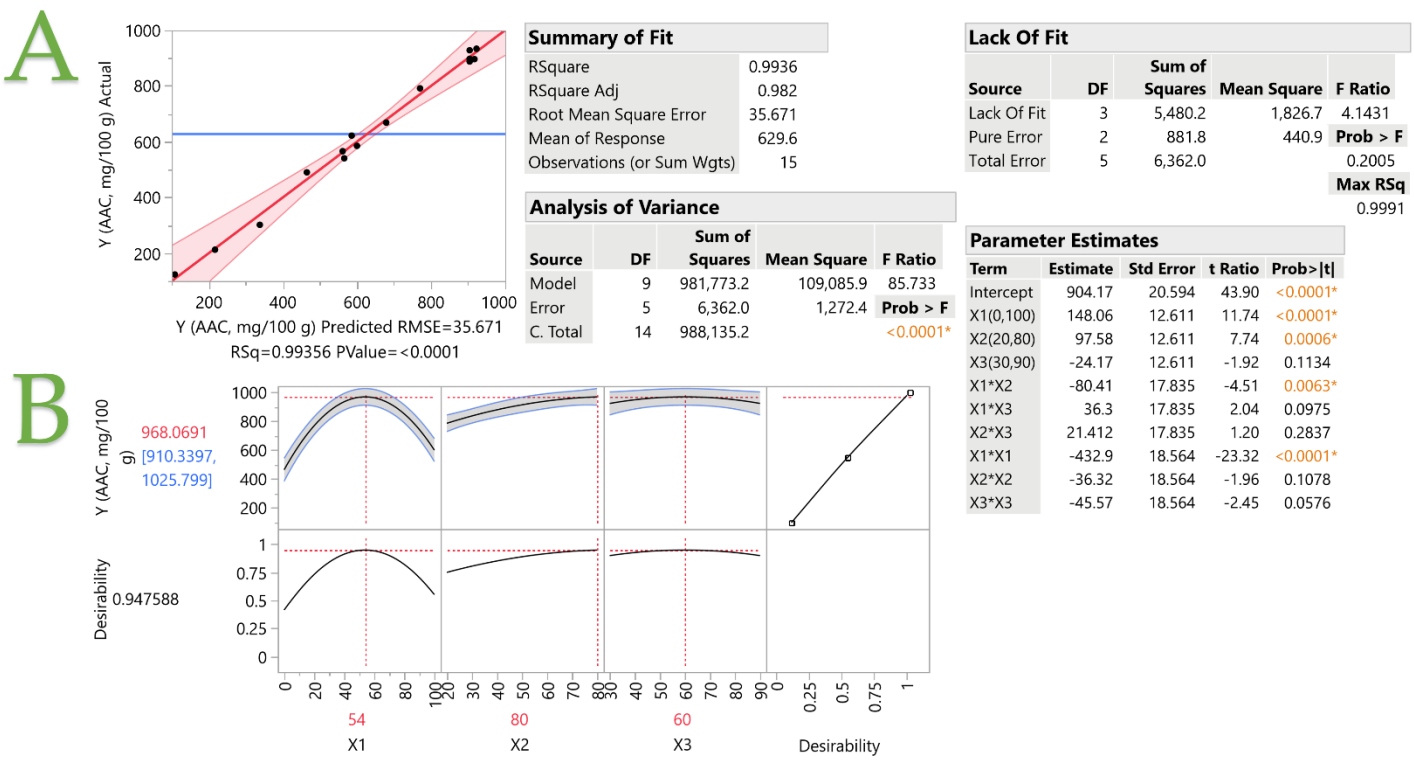


Figure S5. Plot **A** displays the actual response versus the predicted response (Ascorbic acid content – AAC, mg/100 g) for the optimization of PS fruit extracts using hydroethanolic solutions, and different extraction parameters, and plot **B** displays the desirability function. Asterisks and colored values denote statistically significant values, while inset tables include statistics relevant to the evaluation of the resulting model.

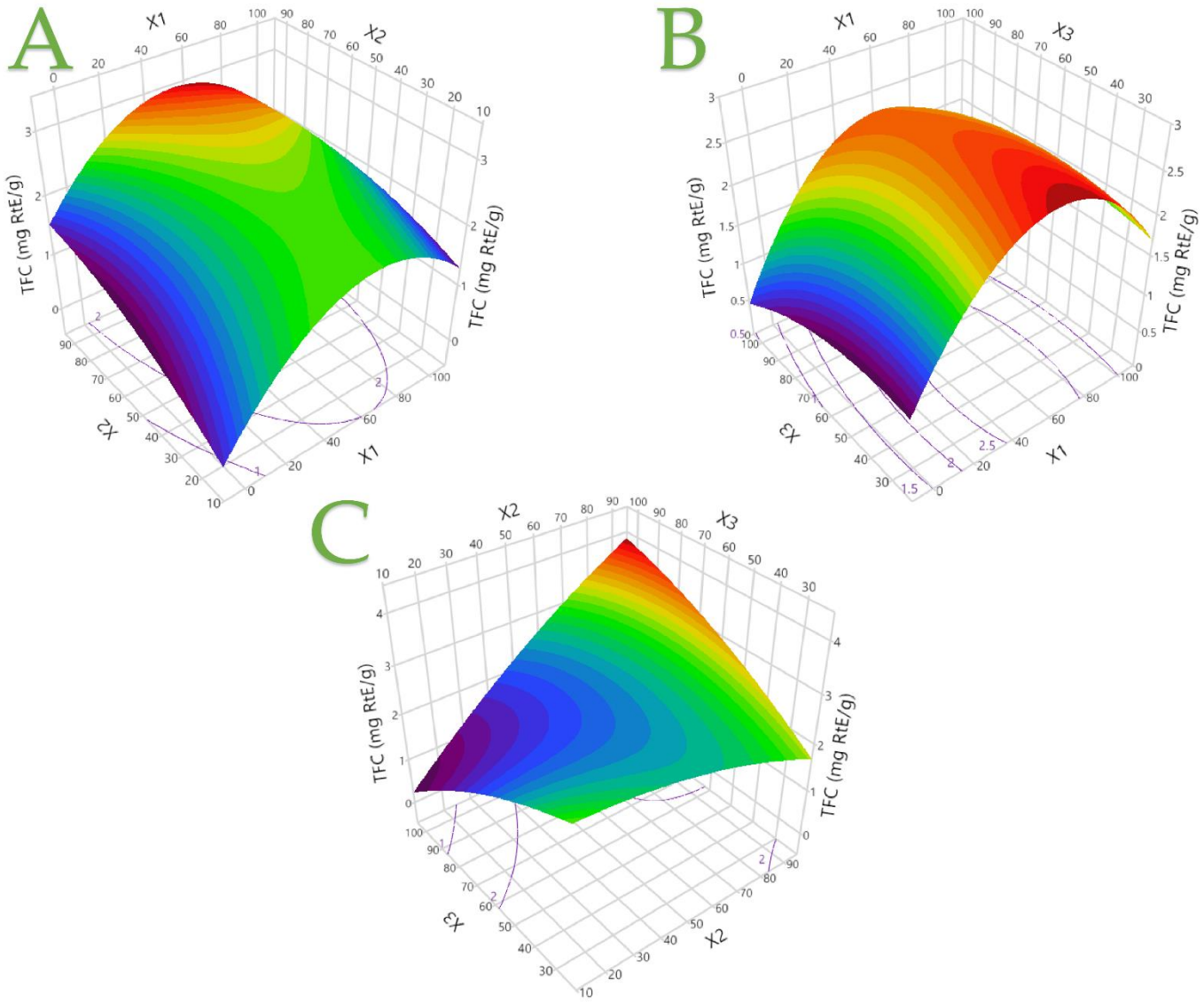


Figure S6. The optimal extraction of PS fruit extracts is shown in 3D graphs that show the impact of the process variables considered in the response (Total flavonoid content – TFC, mg RtE/g). Plot (**A**), covariation of X_1 and X_2; plot (**B**), covariation of X_1 and X_3; plot (**C**), covariation of X_2 and X_3.

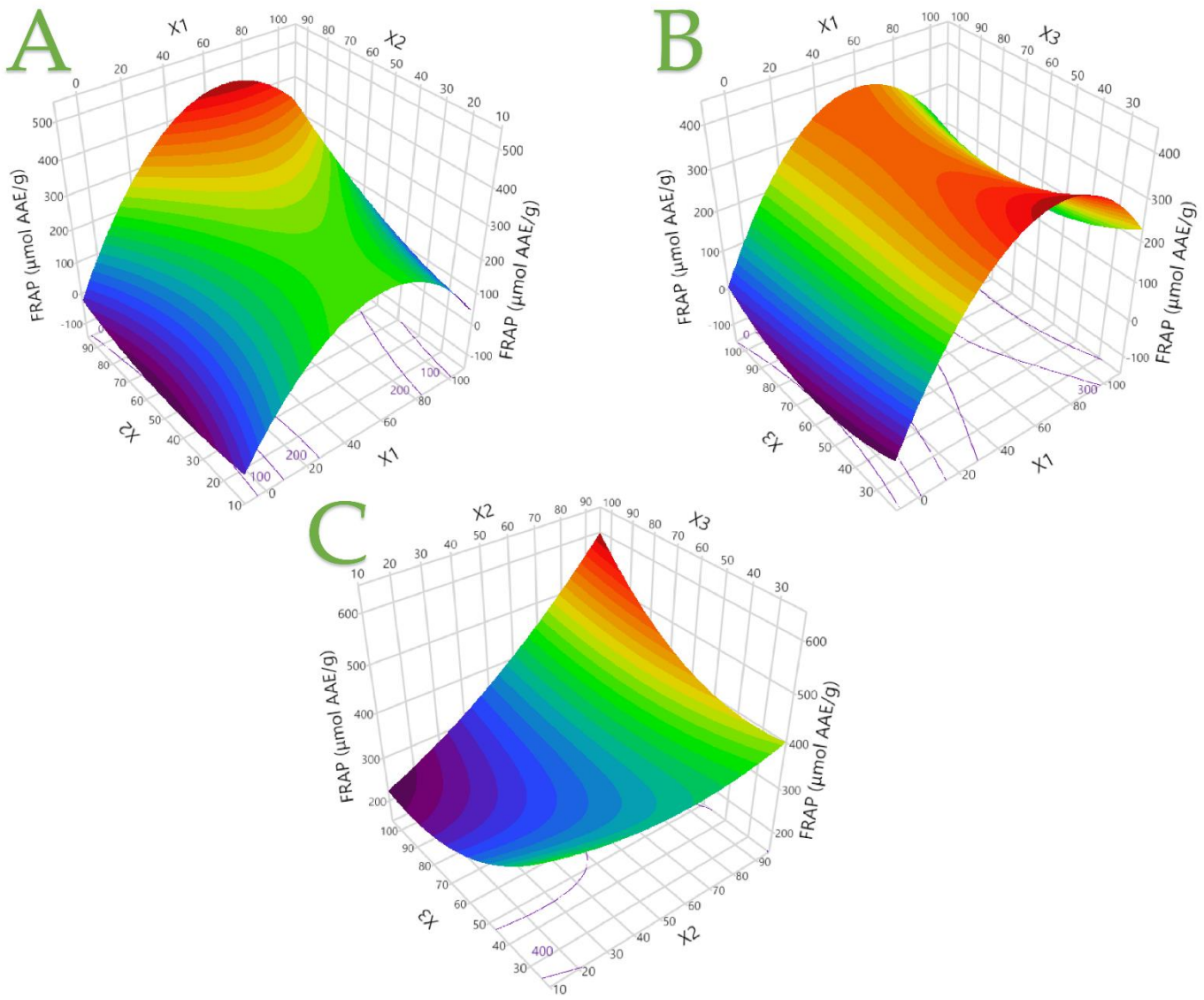


Figure S7. The optimal extraction of PS fruit extracts is shown in 3D graphs that show the impact of the process variables considered in the response (FRAP, µmol AAE/g). Plot (**A**), covariation of X_1 and X_2; plot (**B**), covariation of X_1 and X_3; plot (**C**), covariation of X_2 and X_3.

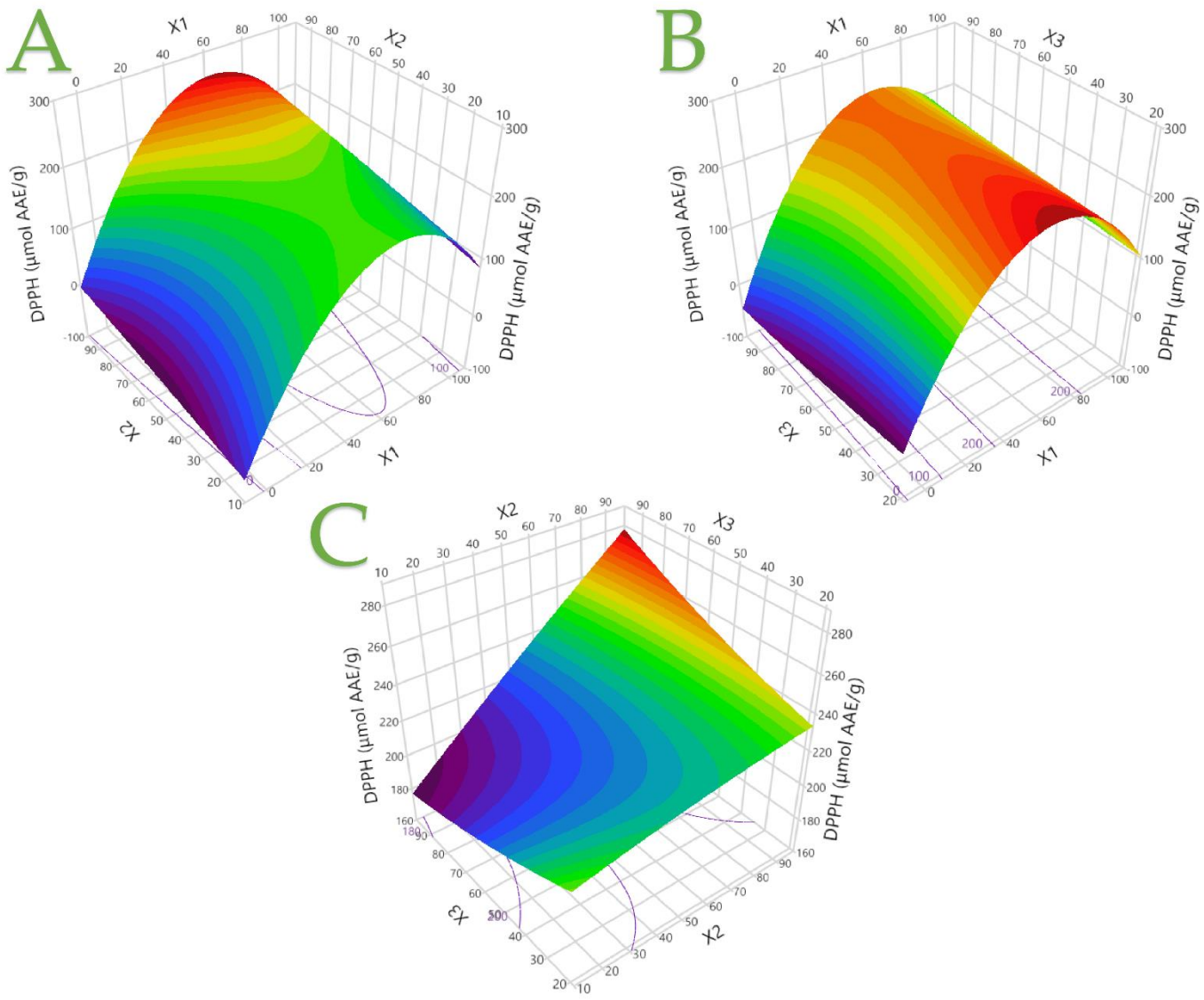


Figure S8. The optimal extraction of PS fruit extracts is shown in 3D graphs that show the impact of the process variables considered in the response (DPPH, µmol AAE/g). Plot (**A**), covariation of X_1 and X_2; plot (**B**), covariation of X_1 and X_3; plot (**C**), covariation of X_2 and X_3.

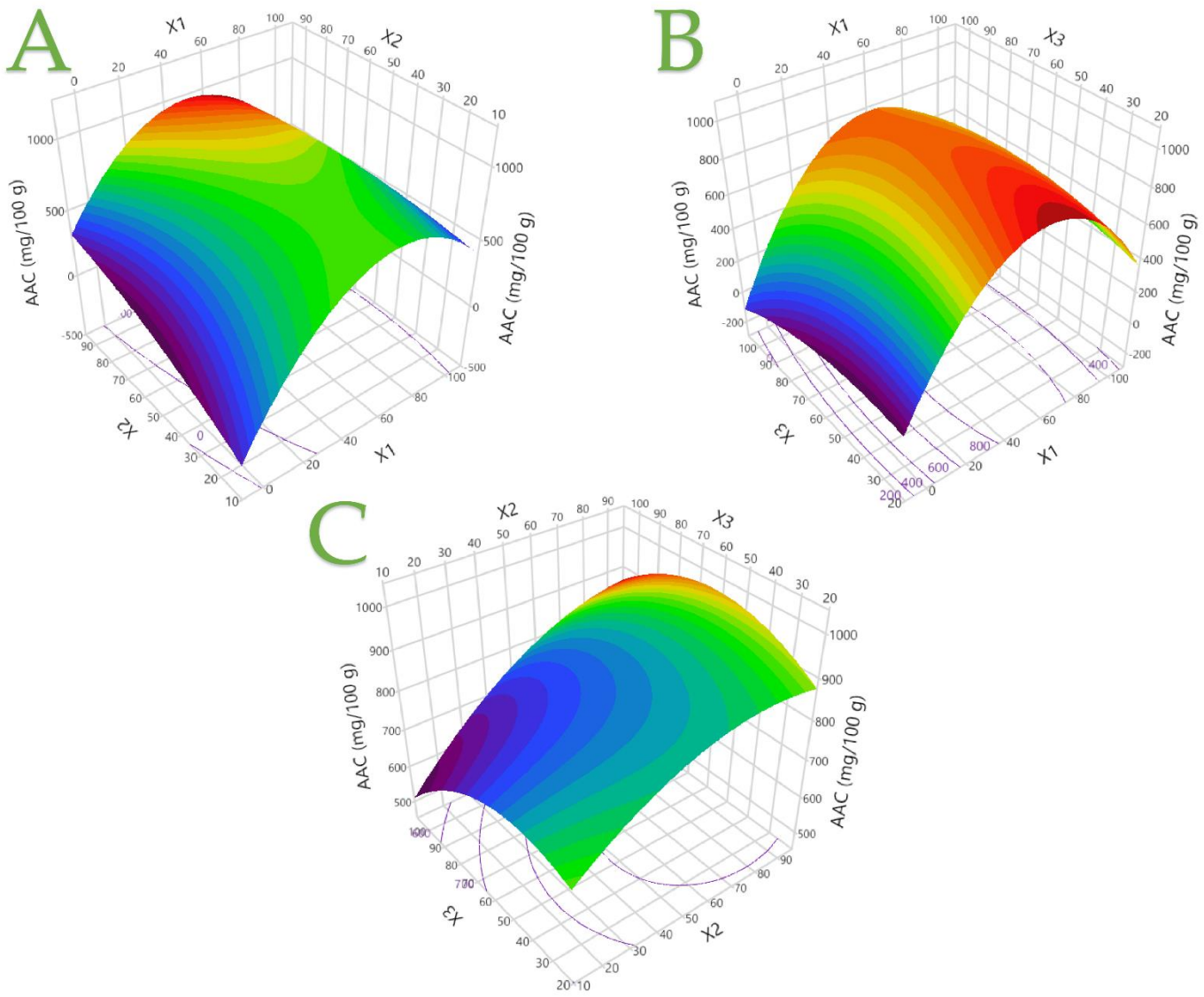


Figure S9. The optimal extraction of PS fruit extracts is shown in 3D graphs that show the impact of the process variables considered in the response (Ascorbic acid content – AAC, mg/100 g). Plot (**A**), covariation of X_1 and X_2; plot (**B**), covariation of X_1 and X_3; plot (**C**), covariation of X_2 and X_3.