Supplementary material

Bioactive Compound Extraction of Hemp (*Cannabis sativa* L.) Leaves through Response Surface Methodology Optimization

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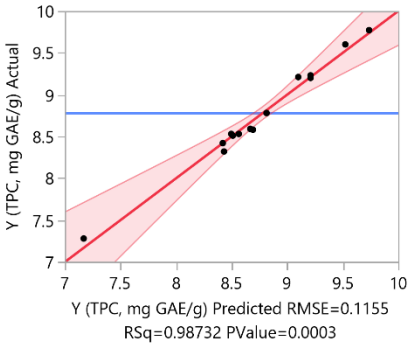
A



Summary of Fit

RSquare	0.9873
RSquare Adj	0.9645
Root Mean Square Error	0.1155
Mean of Response	8.7833
Observations (or Sum Wgts)	15

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	9	5.1911	0.5768	43.2534
Error	5	0.0667	0.0133	Prob > F
C. Total	14	5.2577		0.0003*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	8.2883	0.1163	71.28	<0.0001*
X1(20,50)	0.0263	0.0027	9.67	0.0002*
X2(20,80)	0.2363	0.0408	5.79	0.0022*
X3(30,150)	-0.191	0.0408	-4.68	0.0054*
X1*X2	-0.028	0.0038	-7.40	0.0007*
X1*X3	-0.008	0.0038	-2.21	0.0783
X2*X3	-0.315	0.0577	-5.46	0.0028*
X1*X1	-0.002	0.0003	-6.16	0.0016*
X2*X2	-0.613	0.0601	-10.19	0.0002*
X3*X3	0.1825	0.0601	3.04	0.0289*

B

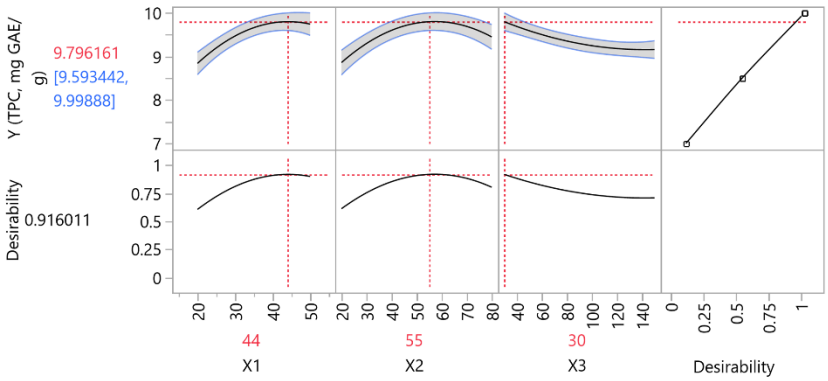


Figure S1. Plots **A** and **B** display the actual response versus the predicted response (Total polyphenol content – TPC, mg GAE/g) for the optimization of *C. sativa* extracts carried out with water solutions, different extraction parameters, and the desirability function. Asterisks and colored values denote statistically significant values, while inset tables include statistics relevant to the evaluation of the resulting model.

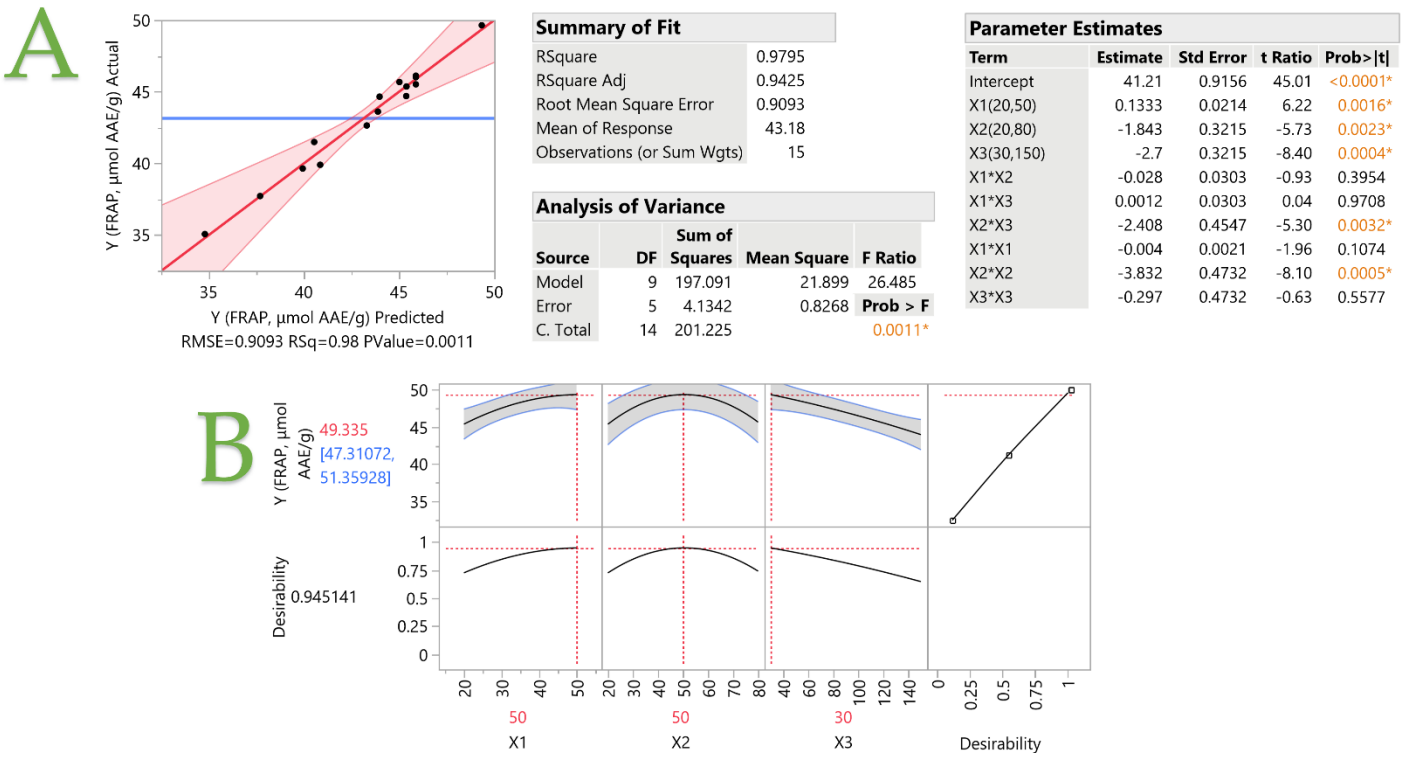


Figure S2. Plots **A** and **B** display the actual response versus the predicted response (FRAP, µmol AAE/g) for the optimization of *C. sativa* extracts carried out with water solutions, different extraction parameters, and the desirability function. Asterisks and colored values denote statistically significant values, while inset tables include statistics relevant to the evaluation of the resulting model.

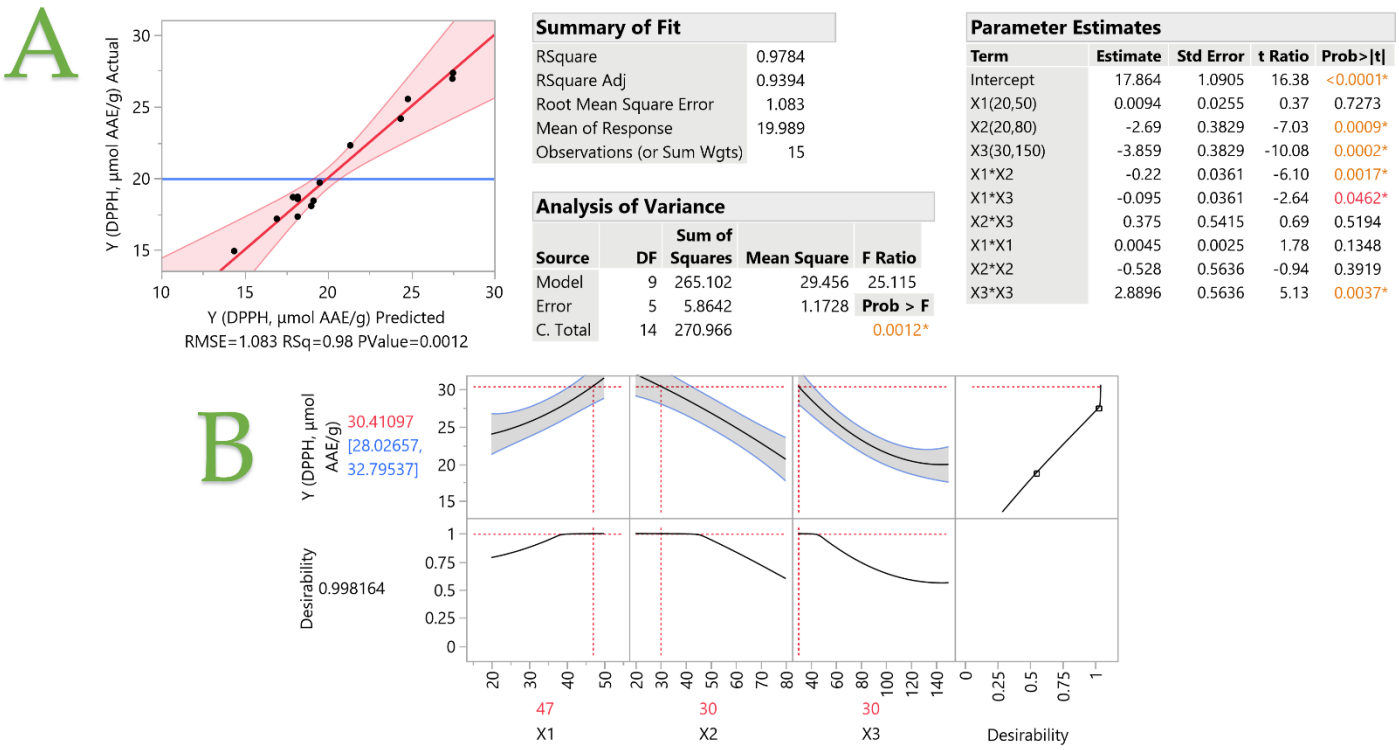


Figure S3. Plots **A** and **B** display the actual response versus the predicted response (DPPH, µmol AAE/g) for the optimization of *C. sativa* extracts carried out with water solutions, different extraction parameters, and the desirability function. Asterisks and colored values denote statistically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

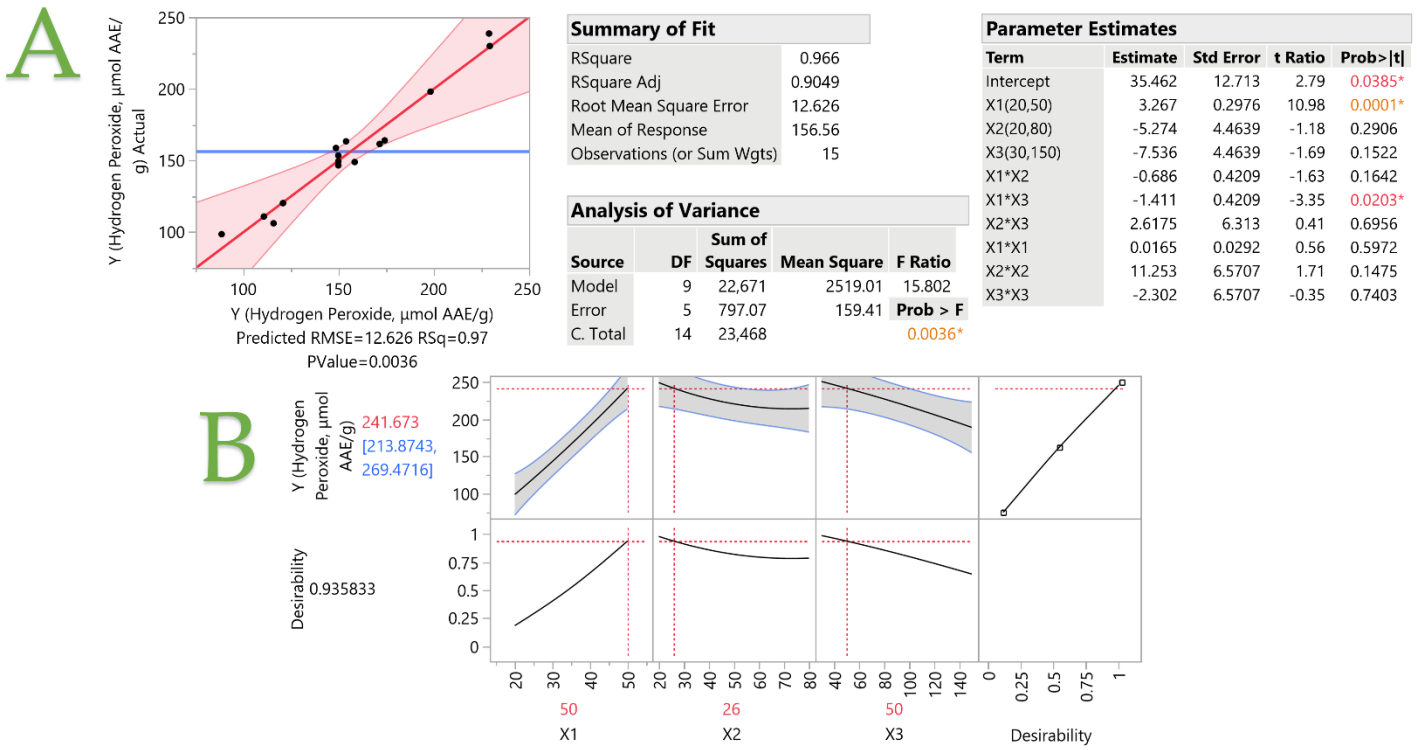


Figure S4. Plots **A** and **B** display the actual response versus the predicted response (Hydrogen Peroxide, µmol AAE/g) for the optimization of *C. sativa* extracts carried out with water solutions, different extraction parameters, and the desirability function. Asterisks and colored values denote statistically significant values, while inset tables include statistics relevant to the evaluation of the resulting model.

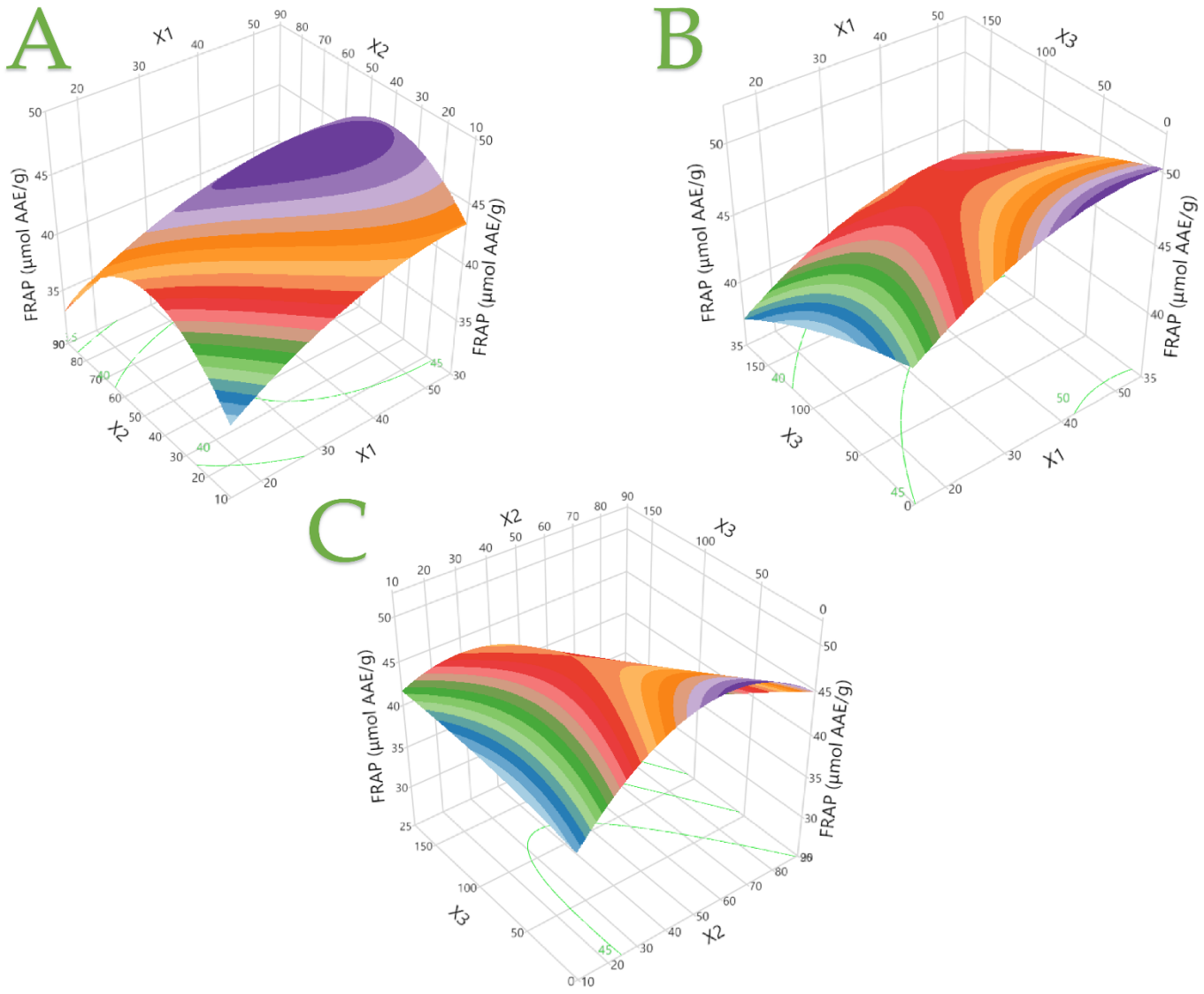


Figure S5. The optimal extraction of *C. sativa* extracts is shown in 3D graphs that show the impact of the process variables considered in the response (FRAP, µmol AAE/g). Plot (**A**), covariation of X_1 and X_2; plot (**B**), covariation of X_1 and X_3; plot (**C**), covariation of X_2 and X_3.

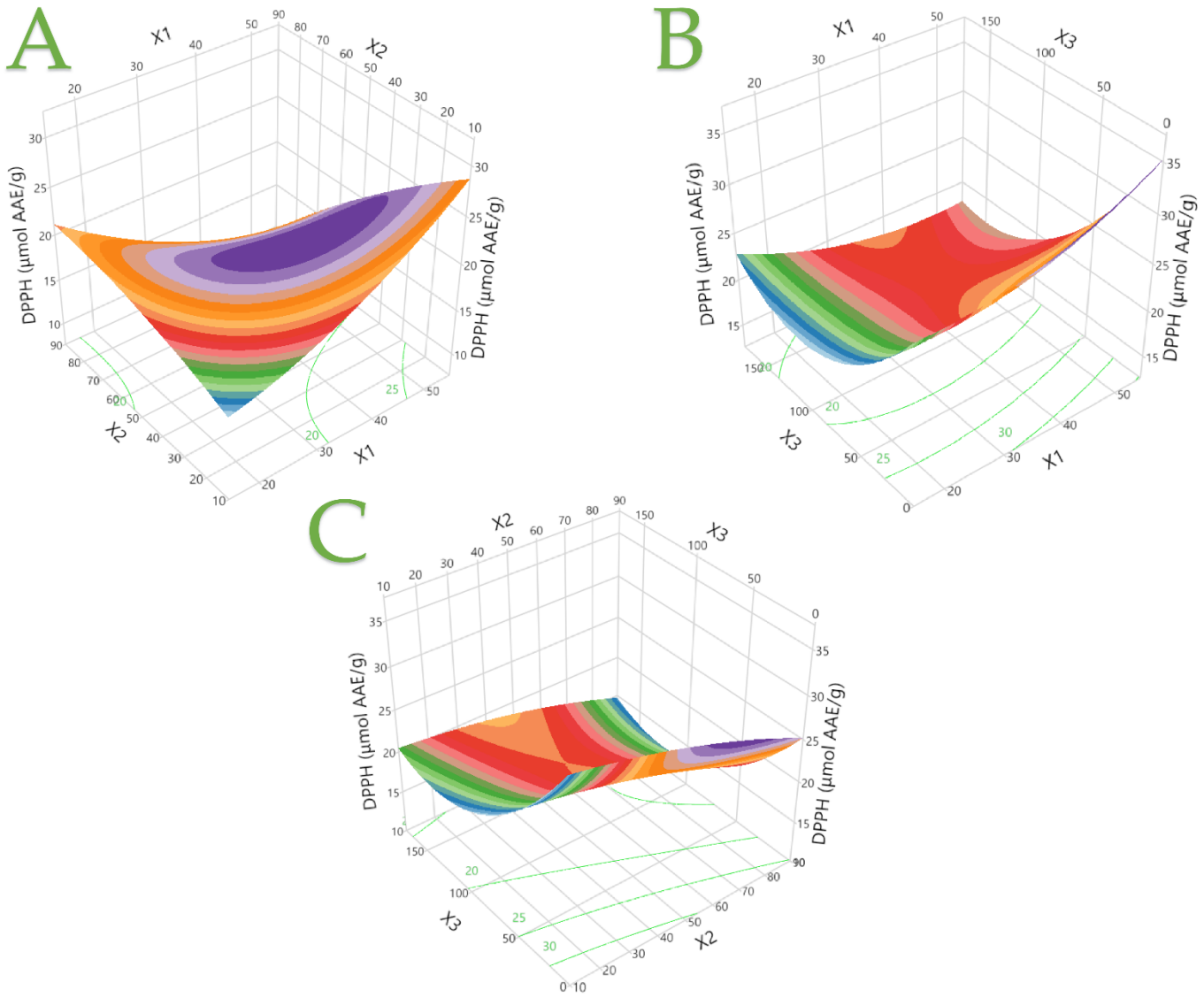


Figure S6. The optimal extraction of *C. sativa* extracts is shown in 3D graphs that show the impact of the process variables considered in the response (DPPH, µmol AAE/g). Plot (**A**), covariation of X_1 and X_2; plot (**B**), covariation of X_1 and X_3; plot (**C**), covariation of X_2 and X_3.

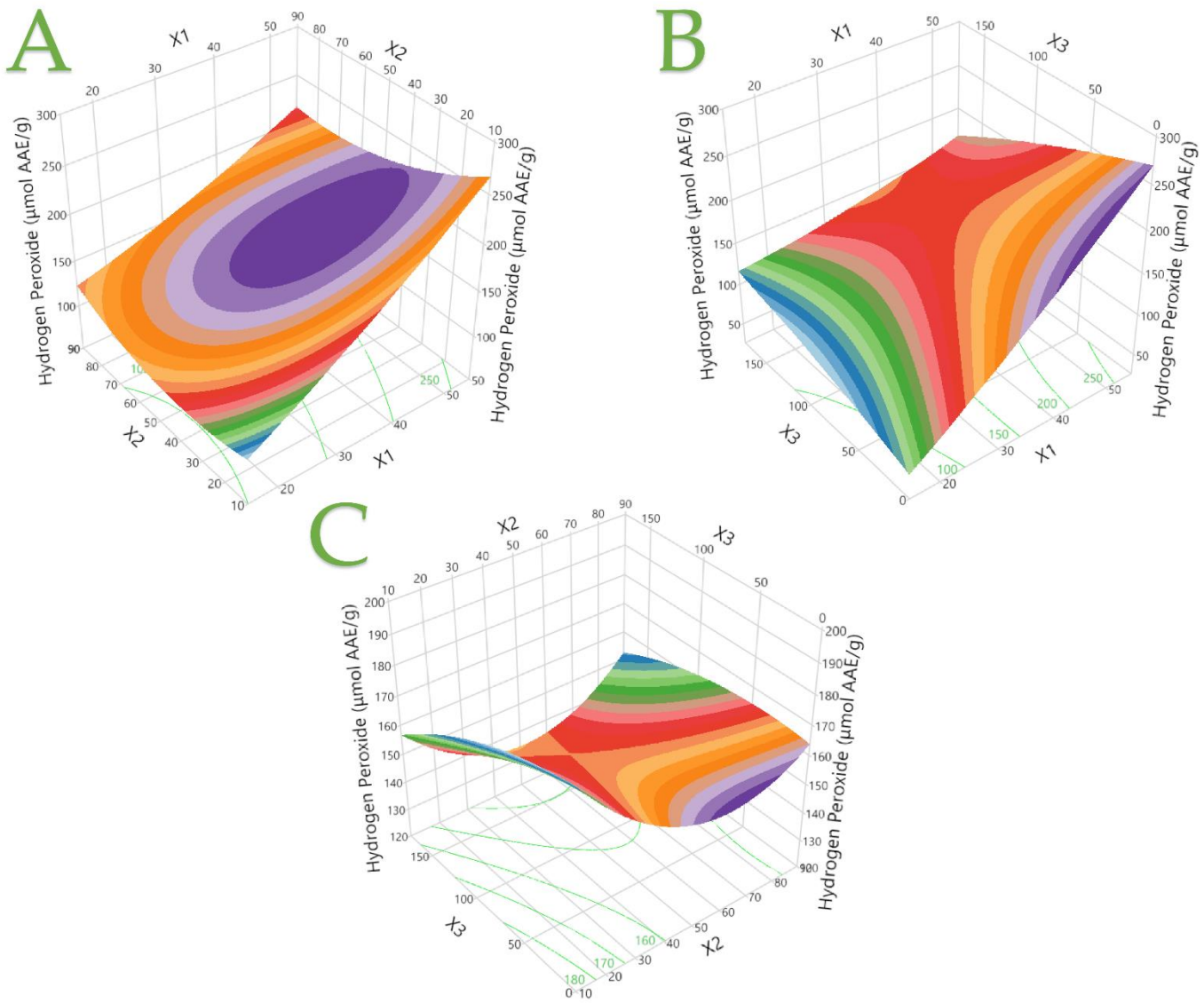


Figure S7. The optimal extraction of *C. sativa* extracts is shown in 3D graphs that show the impact of the process variables considered in the response (Hydrogen Peroxide, µmol AAE/g). Plot (**A**), covariation of X_1 and X_2; plot (**B**), covariation of X_1 and X_3; plot (**C**), covariation of X_2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