

Supplementary Materials

Table S1. BBD with the observed responses and predicted values for TPC.

Run	X ₁	X ₂	X ₃	Total Polyphenolic Content [mg GAE/g]	
				Experimental	Predicted
E1	-1	-1	0	32,91	30,25
E2	1	-1	0	25,63	25,8
E3	-1	1	0	32,14	31,97
E4	1	1	0	26,9	29,57
E5	-1	0	-1	32,88	36,91
E6	1	0	-1	25,4	26,59
E7	-1	0	1	41,47	40,27
E8	1	0	1	47,77	43,74
E9	0	-1	-1	55,75	54,38
E10	0	1	-1	42,19	38,32
E11	0	-1	1	41,96	45,83
E12	0	1	1	66,01	67,38
E13	0	0	0	63,82	62,7
E14	0	0	0	63,76	62,7
E15	0	0	0	60,52	62,7

Table S2. ANOVA for response surface models: estimated regression model of relationship between response variable (TPC) and independent variables (X₁,X₂,X₃) from pomegranate flowers.

Source	DF	Sum of Squeres	F-Value	P-Value
TPC				
Model	9	3070,051	18,29	0,003*
X ₁	1	23,457	1,296	0,306
X ₂	1	15,072	0,834	0,403
X ₃	1	210,197	11,616	0,019*
X ₁ X ₁	1	2118,192	117,0519	0,0001*
X ₂ X ₂	1	323,023	17,85	0,008*
X ₃ X ₃	1	12,895	14,91	0,437
X ₁ X ₂	1	1,051	0,058	0,819
X ₁ X ₃	1	47,497	2,625	0,166
X ₂ X ₂	1	90,481	19,539	0,007

Equation S1 Equation representing the quadratic model fit determination of the sum of polyphenols in pomegranate flowers

$$\text{TPC [mg GAE/g]} = 31,61783 + 0,76753 X_1 + 0,62915 X_2 - 0,51789 X_3 - 0,00958 X_1^2 - 0,01497 X_2^2 - 0,00831 X_3^2 - 0,00041 X_1 X_2 - 0,00459 X_1 X_3 + 0,02507 X_2 X_3$$

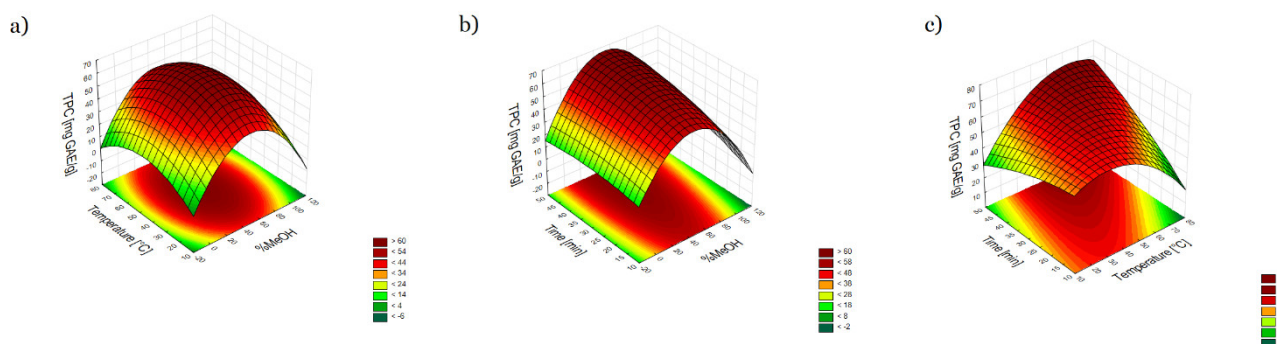


Figure S1 Response surface and contour plots TPC as a function of X_1 and X_3 (a), X_1 and X_2 (b), X_2 and X_3 .

Interpretation

Based on the results, there is a good model fit ($p < 0.05$). The polyphenol content of the raw material is influenced by all the study factors - methanol content, temperature and time. The highest amount of polyphenols is obtained during extraction with 55% methanol at 53 degrees for 43 min.

Methods

The determination of total polyphenols was performed according to the methodology of Kikowska et al. To 25 μL of extracts or gallic acid solution (in the concentration range of 6.25-100 $\mu\text{g/mL}$), 200 μL of distilled water, 15 μL of Folin-Ciocalteu reagent, and 60 μL of 20% sodium carbonate solution were added. Under dark conditions, the plate was shaken for 5 min at room temperature at 600 rpm and then incubated for another 25 min. Five replicates were used for analysis. The total gallic acid content of the extracts obtained was estimated using the calibration curve of the standard substance and presented in milligrams of gallic acid equivalents (GAE) per 1 g of plant material.