RESPONSE SURFACE METHODOLOGY USED FOR THE OPTIMIZATION OF THE FLAVONOID EXTRACT OBTAINING TECHNOLOGICAL PROCESS♦

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Abstract: The objective of this work was to use Box – Wilson experimental plan to establish the relation between two dependent variables (*Y1* (total polyphenols) and *Y2* (antioxidant capacity mM TROLOX/g extract)) and independent variables *X1* (vegetal product/solvent ratio), and *X2* (hydrolysis time) in order to obtain the optimal formula of the technological process using the response surface methodology (RSM). The optimal formula represents one or two formula (solutions of the system) that analyzed the correspondence with the predicted values. To calculate the coefficients for the response equation we used a software running under Excel. For the optimal formula of the technological process we used Matlab 6.5 version (for plots) and for superimposing the Adobe Photosmart – Acrobat Reader software. Statistics ("t" Student test) were generated through the Excel software. The estimation error values prove the validity of

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the mathematical method used. The vegetal product/solvent ratio (mass/volume) is 9.25, the hydrolysis time is 18 hours. These solutions correspond to the optimal value and are statistically validated. The software used is running under Windows and could be used for other formulations as well.

Keywords: *experimental design, optimization, flavonoid extract obtaining*

INTRODUCTION

Response Surface Methodology (RSM) represents a set of statistical and mathematical methods used for analyzing the relation between one or more measured answers (dependent variables) and a number of independent variables, in order to obtain an optimal formula. We applied RSM in order to obtain the optimal formula of the experimental conditions which minimize or maximize a response of the system and the modifications of response surface in the domain of independent variables determination. Starting from these information we optimized the obtaining of a flavonoid extract using a Box – Wilson experimental plan, which established the relation between two dependent variables (*Y1* (total polyphenols) and *Y2* (antioxidant capacity mM TROLOX/g extract) and independent variables *X1* (vegetal product/solvent ratio), and *X2* (hydrolysis time) in order to obtain the optimal formula of the technological process using the response surface methodology [1 - 4].

MATERIALS AND METHODS

The flavonoid extracts were obtained from *Sophorae flos* buds using reflux method with methanol 40%, the methanolic extractive solutions were concentrated by distillation, than hydrolyzed with HCl 2N (different times). After that are extracted with ethyl acetate, dried on anhydrous sodium sulfate, distilled in a rotary evaporator unit until a dry residue is obtained.

We used a Box – Wilson experimental plan with independent variables *Xi* – ratio vegetal product/solvent = X_1, hydrolysis time (h) = X_2, dependent variables – total polyphenols (μM) = *Y1*, antioxidant capacity mM TROLOX/g extract = *Y2*. Using these variables we determined the experiment matrix. To calculate the coefficients for the response equation we used a software running under Excel. For the optimal formula of the technological process we used Matlab 6.5 version (for plots) and for the superposed isoresponse surfaces the Adobe Photosmart – Acrobat Reader software. Statistics ("t" Student test) were generated by the Excel software [5, 6].

RESULTS AND DISCUSSION

Table 1 presents the system matrix; the response coefficients are presented in Table 2, and their values in Table 3. Table 4 presents the "t" Student test values. With these values we draw the three-dimensional response charts (Figures 1 and 2), and by superposing them we can see the optimal zones (Figures 3 and 4). From these charts it may be observed that the total polyphenol content ant the antioxidant capacity of the extracts modifies with the *X1* and *X2* values augmentation. We obtained the isoresponse surfaces which prove the interrelation between these analyzed factors.

Table 1. *Program for optimization the flavonoids extract obtaining process*

Y	*B0*	*B1*	*B2*	*0*	*B4*	*0*	*0*	*B7*	*B8*	*0*	*0*	→	
Y		*X1*	*X2*	*X3*	*X1X2*	*X1X3*	*X2X3*	*X1^2*	*X2^2*	*X1X2X3*	*X3^2*		
No.	*X1*	*X2*	*X3*	*X1X2*	*X1X3*	*X2X3*	*X1^2*	*X2^2*	*X3^2*	*X1X2X3*	*Y1*	*Y2*	*Y3*
1	-1	-1	0	1	0	0	1	1	0	0	3732.16	20.1	0
2	1	-1	0	-1	0	0	1	1	0	0	3585.18	20.36	0
3	-1	1	0	-1	0	0	1	1	0	0	3374.12	19.45	0
4	1	1	0	1	0	0	1	1	0	0	3118.42	17.06	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0.00	0	0	0	0	0	0	0	0	0	0	0	0
10	0.00	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0.00	0	0	0	0	0	0	0	0	0	0	0
12	0	0.00	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0.00	0	0	0	0	0	0	0	0	0	0
14	0	0	0.00	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0
											13809.88	76.97	0

Table 2. *System matrix, response coefficients*

No	**B0**	**B1**	**B2**	**0**	**B4**	**0**	**0**	**B7**	**B8**	**0**	**0**
1	1	-1	-1	-1	1	1	1	1	1	1	1
2	1	1	-1	-1	-1	-1	1	1	1	-1	1
3	1	-1	1	-1	-1	1	-1	1	1	-1	1
4	1	1	1	-1	1	-1	-1	1	1	1	1
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0
Y1	3452.47	-100.67	-206.20	0.000	-27.180	0.000	0.000	100.67	1893.26	0.000	0,000
Y2	19.243	-0.533	-0.988	0.000	-0.663	0.000	0.000	0.533	0.988	0.000	0,000
Y3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0,000
	B0	B1	B2	0.000	B4	0.000	0.000	B7	B8	0.0	0,000
	OK	**OK**	**OK**	**Attn!**	**OK**	**Attn!**	**Attn!**	**OK**	**OK**	**Attn!**	**Attn!**

Table 3. *System matrix, response coefficients values*

COVARIANCE									
Y1	-26.845	-54.987	0.000	-7.248	0.000	0.000	675.150	675.150	0.000
Y2	-0.142	-0.263	0.000	-0.177	0.000	0.000	3.763	3.763	0.000
Y3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 4. *"t" Student test values*

"t"Student test							
No.	X1	X2	X3	Y1	Y2	Y3	
1	5.00	8.00	0.00	3732.16	20.1	0	
2	10.00	16.00	0.00	3585.18	20.36	0	0.490
3	12.00	20.00	0.00	3374.12	19.45	0	0.484
4	15.00	24.00	0.00	3118.42	17.06	0	0.479
5	0.00	0.00	0.00	0	0	0	0.177
6	0.00	0.00	0.00	0	0	0	#DIV/0!
7	0.00	0.00	0.00	0	0	0	#DIV/0!
8	0.00	0.00	0.00	0	0	0	#DIV/0!
9	0.00	0.00	0.00	0	0	0	#DIV/0!
10	0.00	0.00	0.00	0	0	0	#DIV/0!
11	0.00	0.00	0.00	0	0	0	#DIV/0!
12	0.00	0.00	0.00	0	0	0	#DIV/0!
13	0.00	0.00	0.00	0	0	0	#DIV/0!
14	0.00	0.00	0.00	0	0	0	#DIV/0!
15	0.00	0.00	0.00	0	0	0	#DIV/0!
			No.	X1	X2	X3	
			→	5.00	8.00	0.00	
			2	10.00	16.00	0.00	
			3	12.00	20.00	0.00	
			4	15.00	24.00	0.00	
			5	0.00	0.00	0.00	
			6	0.00	0.00	0.00	
			7	0.00	0.00	0.00	
			8	0.00	0.00	0.00	
			9	0.00	0.00	0.00	
			10	0.00	0.00	0.00	
			11	0.00	0.00	0.00	
			12	0.00	0.00	0.00	
			13	0.00	0.00	0.00	
			14	0.00	0.00	0.00	
			→	0.00	0.00	0.00	

The optimal formula for flavonoid extract obtaining with the greatest content in total polyphenols and high antioxidant capacity are presented in Figure 5 and Table 5.
The response equations are:

$$Y1 = 3452.470 - 100X_1 - 0.206X_2 - 27.18X_3 + 100.67X_1X_3 + 1893.26X_2X_3 \quad (1)$$

$$Y2 = 19.24 - 0.533X_1 - 0.988X_2 - 0.663X_3 + 0.533X_1X_3 + 0.899X_2X_3 \quad (2)$$

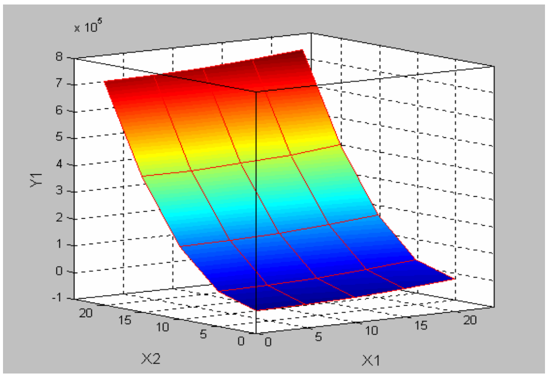

Figure 1. Response surface for variable Y1/ X1 - X2

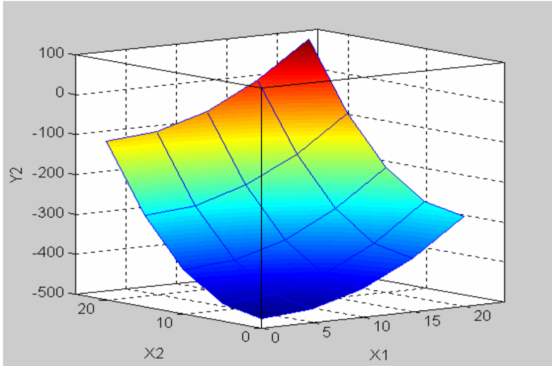

Figure 2. Response surface for variable Y2/ X2 – X1

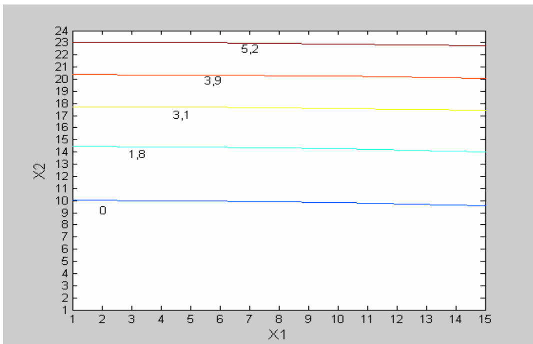

Figure 3. Isoresponce surface for variable Y2/ X1 - X2

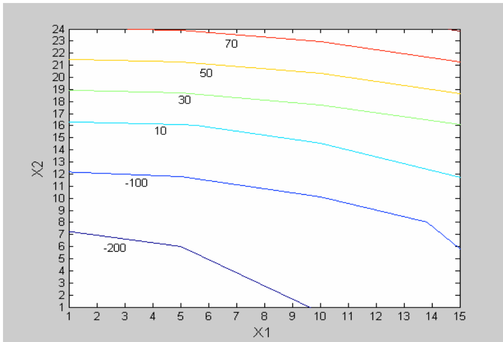

Figure 4. Isoresponce surface for variable Y1/ X1 - X2

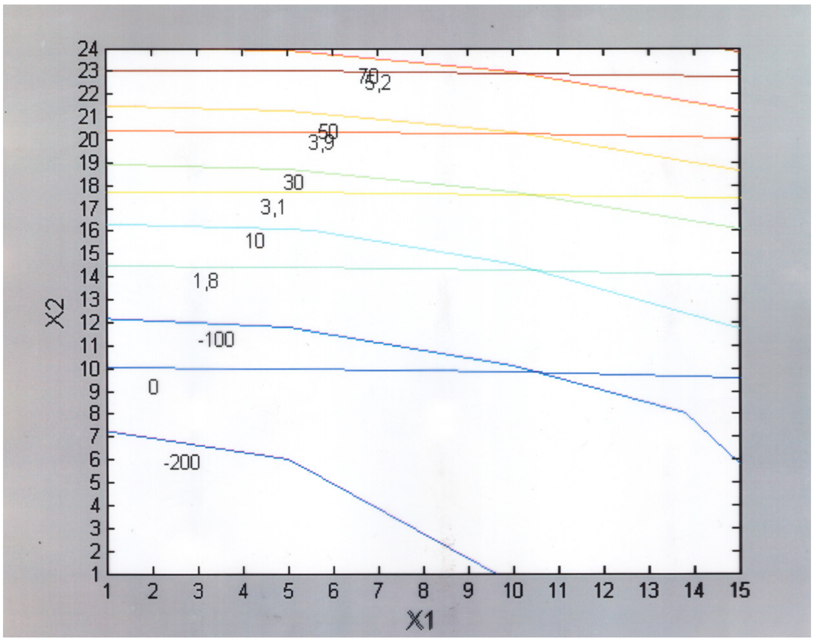

Figure 5. Isoresponce surface superposed

Table 5. *Optimal formula for the used parameters*

Factors	Minimum limits	Maximum limits	Average
Vegetal product/solvent ratio [m/V]	9	9.5	9.25
Hydrolysis time [h]	18	18	18

Based on these values, the comparative results estimated and experimental are presented in table 6.

Table 6. *The comparative results of the optimal formula*

Factors	Utilized values	Experimental values	Estimation error
Vegetal product/solvent ratio [m/V]	10	9.25	+ 0.75
Hydrolysis time [h]	15	18	+ 3

CONCLUSION

The optimal ratio vegetal product/solvent (mass/volume) is 9.25, with a hydrolysis time of 18 hours. These values are statistical validated.

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