Response surface methodology (RSM)

A set of central composite, face centered experiments of full factorial design at two levels, three factors with a total of 20 runs, in a single block, were applied to evaluate the curvature model. The independent variables were temperature, time and solvent/plant ratio, applied to an ultrasonic assisted extraction of dry pomace in water. The effects of each independent variables were extimated by quantitative HPLC of a set of phenolics

Table S1 reports the estimated regression coefficients of the second order polynomial equations for RSM analysis of each metabolite detected in pomace water extract.

Regression coefficients for mean, linear, interaction and quadratic terms, were calculated respectively from the experimental results by the least squares method. Minitab 16 software was used. The ANOVA analysis was applied to evaluate the relevance of independent variables' influence and interactions ($p<0.05$). The adequacy of the model was predicted on the basis of the coefficient of determination (Rsq), the significance (p) and the lack of adjustment tests.

TABLE S1: Estimated regression coefficient of the second order polynomial equation for response surface methodology analysis of secondary metabolite classes extraction (uncoded).

TERM	**Estimated Regression Coefficients**	**P**	**Regression p-value**	**R squared**	**Lack of fit**
Gallic acid					
Constant	2.52718	0.023	0.001	88.54%	0.574
Temperature (°C)	0.00299769	0.143			
Time (min)	0.0285591	0.000			
Solvent/Plant (v/w)	0.0748758	0.808			
Temperature (°C)*Temperature (°C)	4.56397E-05	0.298			
Time (min)*Time (min)	-2.88679E-04	0.031			
Solvent/Plant (v/w)*Solvent/Plant (v/w)	-0.00646757	0.574			
Temperature (°C)*Time (min)	-7.46667E-05	0.633			
Temperature (°C)*Solvent/Plant (v/w)	0.000197917	0.734			
Time (min)*Solvent/Plant (v/w)	-1.68750E-04	0.023			

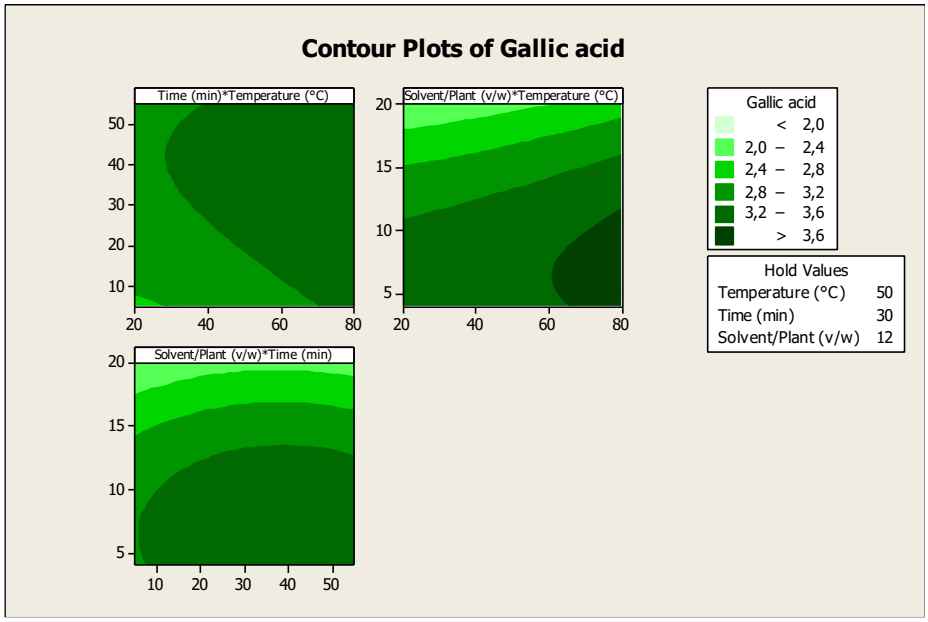
Caftaric acid					
Constant	2.74663	0.722		75.19%	0.969
Temperature (°C)	0.00167682	0.918			
Time (min)	0.0133576	0.322			
Solvent/Plant (v/w)	0.124634	0.996			
Temperature (°C)*Temperature (°C)	-5.72391E-07	0.301			
Time (min)*Time (min)	-1.69624E-04	0.009			
Solvent/Plant (v/w)*Solvent/Plant (v/w)	-0.00489086	0.577			
Temperature (°C)*Time (min)	-4.38333E-05	0.913			
Temperature (°C)*Solvent/Plant (v/w)	2.65625E-05	0.826			
Time (min)*Solvent/Plant (v/w)	-6.43750E-05	0.722			
Catechin					
Constant	-7.03796	0.000	0.018	78.85%	0.169
Temperature (°C)	0.159237	0.439			
Time (min)	0.198819	0.787			
Solvent/Plant (v/w)	1.16060	0.227			
Temperature (°C)*Temperature (°C)	-0.00123588	0.334			
Time (min)*Time (min)	-0.00282367	0.138			
Solvent/Plant (v/w)*Solvent/Plant (v/w)	-0.0411765	0.037			
Temperature (°C)*Time (min)	-3.75167E-04	0.671			
Temperature (°C)*Solvent/Plant (v/w)	-7.45313E-04	0.786			
Time (min)*Solvent/Plant (v/w)	-0.00141938	0.668			

Chlorogenic acid					
Constant	0.996257	0.038	0.054	72.55%	0.000
Temperature (°C)	0.000429174	0.144			
Time (min)	-5.80623E-04	0.039			
Solvent/Plant (v/w)	0.00576045	0.763			
Temperature (°C)*Temperature (°C)	6.59035E-06	0.616			
Time (min)*Time (min)	1.58901E-05	0.916			
Solvent/Plant (v/w)*Solvent/Plant (v/w)	-3.23232E-05	0.075			
Temperature (°C)*Time (min)	2.97833E-05	0.062			
Temperature (°C)*Solvent/Plant (v/w)	-9.82813E-05	0.099			
Time (min)*Solvent/Plant (v/w)	-1.02063E-04	0.038			
Epicatechin					
Constant	0.855679	0.031	0.030	76.28%	0.471
Temperature (°C)	-0.00153196	0.027			
Time (min)	0.00284779	0.693			
Solvent/Plant (v/w)	0.163681	0.800			
Temperature (°C)*Temperature (°C)	3.47363E-05	0.641			
Time (min)*Time (min)	9.24202E-05	0.006			
Solvent/Plant (v/w)*Solvent/Plant (v/w)	-0.00658183	0.628			
Temperature (°C)*Time (min)	4.70000E-05	0.597			
Temperature (°C)*Solvent/Plant (v/w)	0.000160417	0.343			
Time (min)*Solvent/Plant (v/w)	-3.51250E-04	0.031			

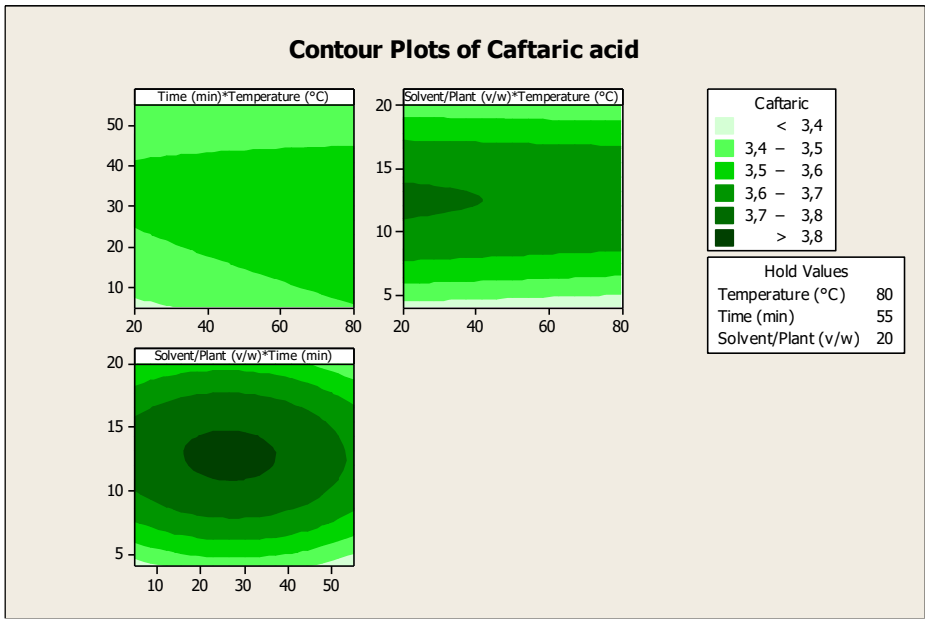
Caffeic acid					
Constant	0.254769	0.336	0.018	78.98%	0.642
Temperature (°C)	-0.00116945	0.112			
Time (min)	0.00346747	0.534			
Solvent/Plant (v/w)	0.0309590	0.599			
Temperature (°C)*Temperature (°C)	1.31987E-05	0.278			
Time (min)*Time (min)	-4.01939E-05	0.004			
Solvent/Plant (v/w)*Solvent/Plant (v/w)	-0.00129877	0.857			
Temperature (°C)*Time (min)	-3.16667E-06	0.617			
Temperature (°C)*Solvent/Plant (v/w)	2.76042E-05	0.902			
Time (min)*Solvent/Plant (v/w)	-8.12500E-06	0.336			
Syringic acid					
Constant	0.656014	0.056	0.018	79.10%	0.641
Temperature (°C)	-0.00382217	0.064			
Time (min)	0.00324294	0.772			
Solvent/Plant (v/w)	0.0431347	0.202			
Temperature (°C)*Temperature (°C)	4.07800E-05	0.436			
Time (min)*Time (min)	-3.48768E-05	0.005			
Solvent/Plant (v/w)*Solvent/Plant (v/w)	-0.00152028	0.106			
Temperature (°C)*Time (min)	3.73333E-05	0.655			
Temperature (°C)*Solvent/Plant (v/w)	-3.02083E-05	0.080			
Time (min)*Solvent/Plant (v/w)	-1.53750E-04	0.056			

The ANOVA analysis of the model for the phenols metabolites extractions from grape shows that the models is significant ($p<0.05$) according to R squared and p- values for gallic acid, syringic acid, caffeic acid, benzoic acid catechin and epicatechin. The missing significance for the lack of fit in the model support the applicability of the model in prediction of selected metabolites recovery. The model highlights a suitability for prediction of catechin recovery (78.85% and P=0.169 as R Sq and Lack of fit. respectively), being catechin the most aboundant metabolite and the quadratic solvent-plant ratio a critical factor.

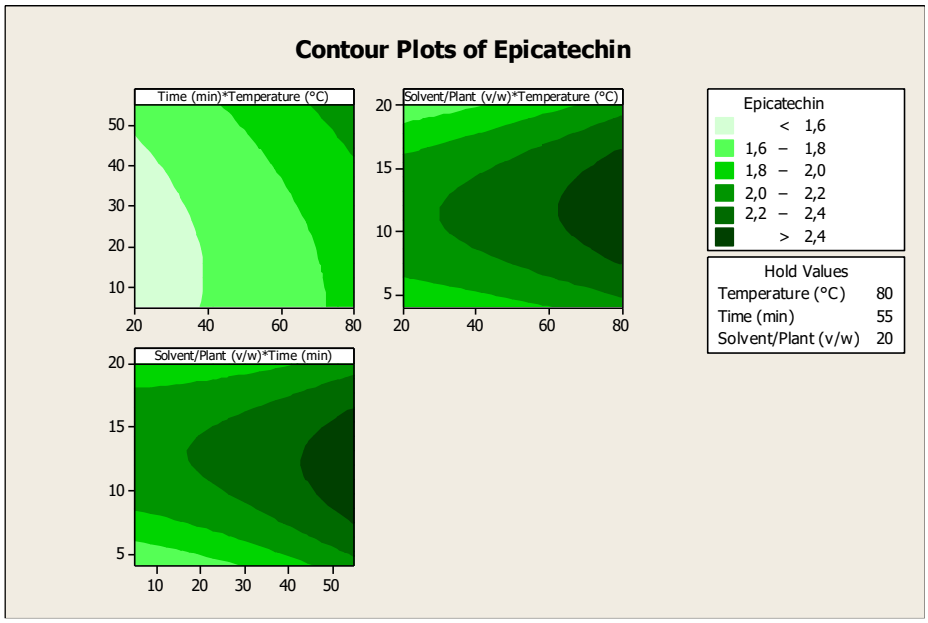
To visualize the relationship between the response and experimental levels of the independent variables for each detected metabolite, the contour plots were constructed according to the quadratic polynomial model equations and reported in Figures S1 A-G.



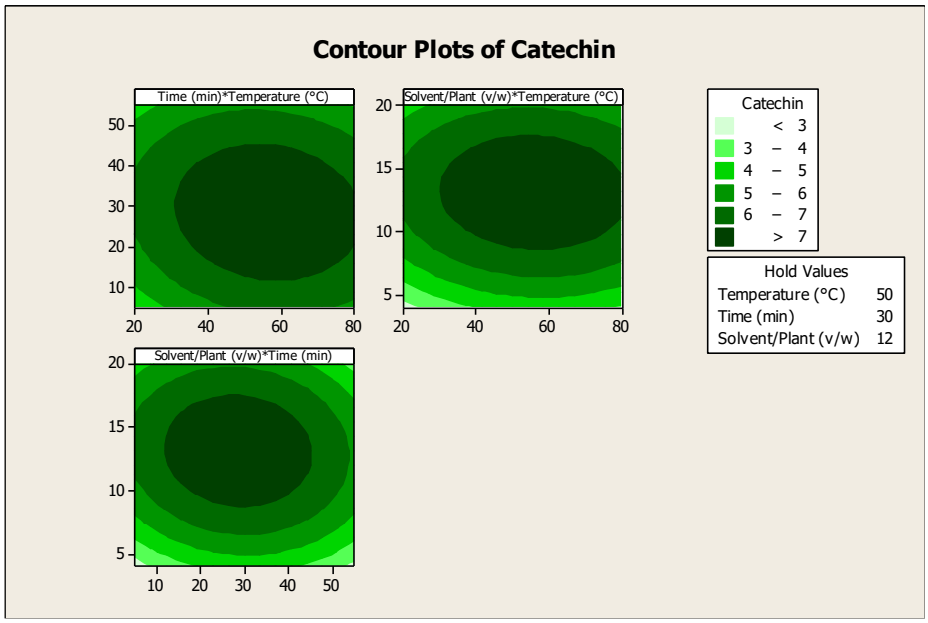
A


Contour Plots of Caftaric acid
Time (min)*Temperature (°C)
Solvent/Plant (v/w)*Temperature (°C)
Solvent/Plant (v/w)*Time (min)
Caftaric
< 3,4
3,4 – 3,5
3,5 – 3,6
3,6 – 3,7
3,7 – 3,8
> 3,8
Hold Values
Temperature (°C) 80
Time (min) 55
Solvent/Plant (v/w) 20

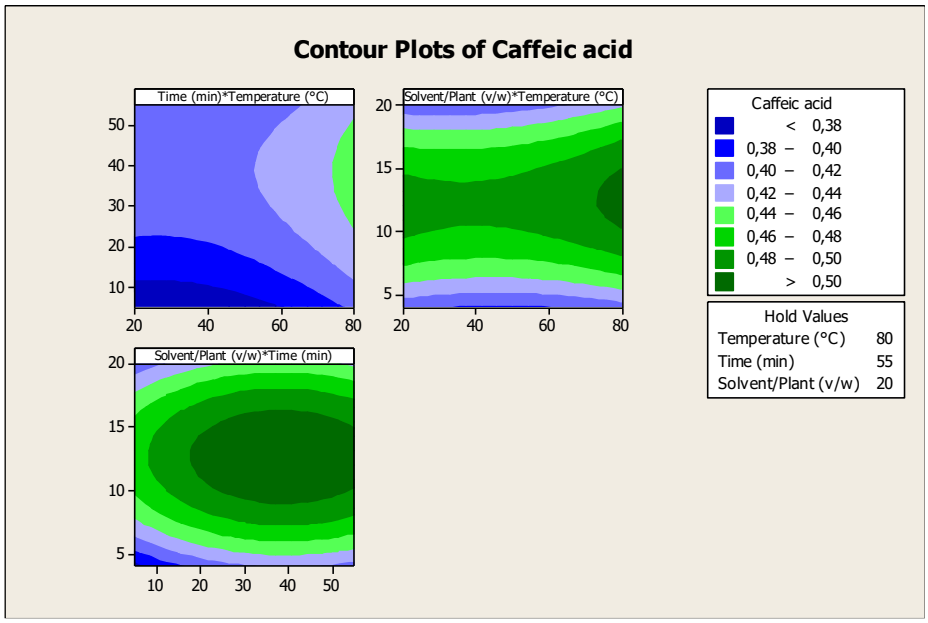
B


Contour Plots of Epicatechin
Time (min)*Temperature (°C)
Solvent/Plant (v/w)*Temperature (°C)
Solvent/Plant (v/w)*Time (min)
Epicatechin
< 1,6
1,6 – 1,8
1,8 – 2,0
2,0 – 2,2
2,2 – 2,4
> 2,4
Hold Values
Temperature (°C) 80
Time (min) 55
Solvent/Plant (v/w) 20

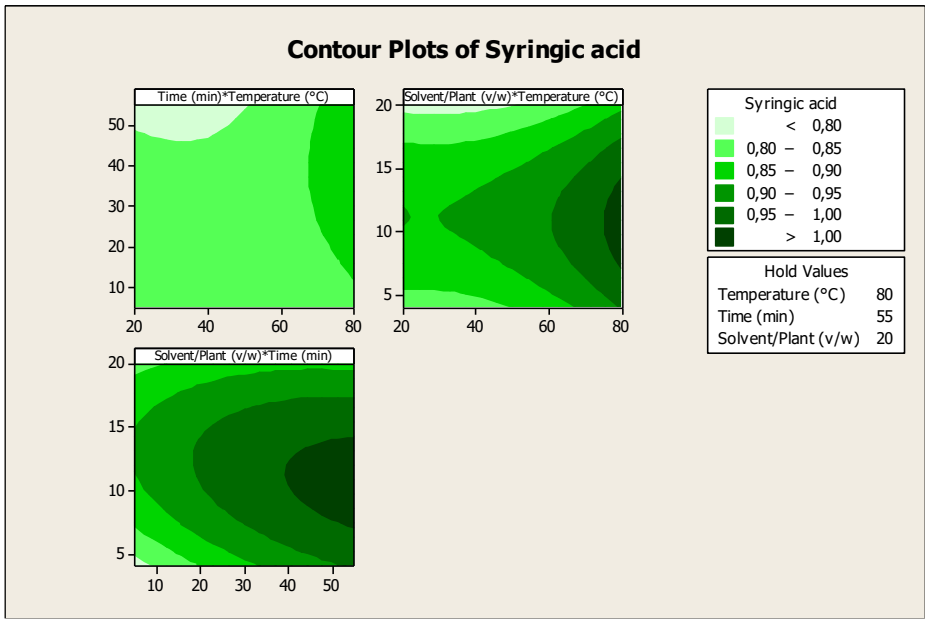
C


Contour Plots of Catechin
Time (min)*Temperature (°C)
Solvent/Plant (v/w)*Temperature (°C)
Solvent/Plant (v/w)*Time (min)
Catechin
< 3
3 – 4
4 – 5
5 – 6
6 – 7
> 7
Hold Values
Temperature (°C) 50
Time (min) 30
Solvent/Plant (v/w) 12

D


Contour Plots of Caffeic acid
Time (min)*Temperature (°C)
Solvent/Plant (v/w)*Temperature (°C)
Solvent/Plant (v/w)*Time (min)
Caffeic acid
< 0,38
0,38 – 0,40
0,40 – 0,42
0,42 – 0,44
0,44 – 0,46
0,46 – 0,48
0,48 – 0,50
> 0,50
Hold Values
Temperature (°C) 80
Time (min) 55
Solvent/Plant (v/w) 20

E



F

Figure S1. Effect of critical factors on phenolic and flavonid extraction (A-F): contour graphs of plant/solvent interaction with other variables at central point level showed the optimal conditions for catechin extraction (30 minutes, 12 Solvent/Plant ratio, temperature 50°C)