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Optimization of extraction parameters of phenolic antioxidants from defatted grape seeds flour by response surface methodology

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Abstract

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Introduction. The optimization results of the conditions of liquid-solid extraction by wine industry waste water in the form of the defatted grape seeds flour in order to obtain the extract with high antioxidant capacity are conducted in this report.

Materials and methods. Total antioxidant capacity (TAC) and total phenolic content (TPC) of the samples were determined by the method of galvanostatic coulometric titration with electrogenerated bromine and spectrophotometric method using Folin-Ciocalteu reagent, respectively. TAC and TPC experimental values were presented in terms of the equivalent of gallic acid content (GAE) per unit mass of dry powder (DW).

Results and discussion. Response surface methodology (RSM) is used to search for optimal condition of solid-liquid extraction of phenolic compounds from defatted grape seeds flour under the influence of three factors: the temperature (60–100 °C), extraction time (90–150 min) and liquid to solid ratio (60-100). The result showed that the phenolic substances yield in the set ranges is 1.20–2.64% with total antioxidant capacity of 17.71–36.78 mg GAE/g DW. Due to optimization procedure, it was determined that under optimal conditions (the temperature of 100 °C; the extraction time is 131 min.; and the ratio of the extractive agent volume to the mass of the powder is 85) the maximum TAC of the extract of 37.04 mg GAE/g DW is achieved. The maximum yield of phenolic substances of 2.646% was obtained under the following conditions: temperature of 100 °C; extraction time is 117 min.; and the ratio of extraction agent volume to the mass of the powder is 93. When optimization is used with two TAC and YPC response functions, the following optimal conditions were obtained: the temperature of 100 °C, the extraction time is 123 minutes and the ratio of extractive agent volume to the mass of powder is 89, at which 36.91 mg GAE/g DW TAC and YPC values and 2.633% were obtained, respectively. The validation of obtained results showed their compliance within 3% with experimental values.

Conclusion. The obtained results indicate the perspective of wine industry waste recycling in order to obtain the solid extract from defatted grape seeds flour as a source of biologically active substances of a phenolic nature with high antioxidant potential.

Introduction

Plant objects are considered to be promising natural sources of antioxidants, and therefore, the number of studies on the development of plant-based additives technologies in the form of pastes, extracts and powders has recently increased. The supplementation of such additives allows obtaining functional nutrition products with high antioxidant potential as well as high biological and nutritional value.

Grape seeds are produced in large amounts as the wine industry waste products and are increasingly used to create food ingredients. This occurs due to the fact that it is a source of polyphenolic antioxidants – flavonoids, such as monomeric flavanols, dimeric, trimeric and polymeric procyanides and phenolic acids [1–3].

The extraction from raw plant materials is an important stage in phytochemical processing in order to optimize the concentration of biologically active compounds. The selection of a suitable solvent for the standardization of plant products is highly important in this process. Differences in the compounds structure determine their solubility in solvents of different polarity. Therefore, the type of extractive solvent can have substantial impact on the yield of the desired extracted compounds from the plant material. However, the selection of solvent is normally limited to water and ethanol or their mixture when using extracts for food purposes.

The selection of an appropriate withdrawal process and the optimization of various parameters are crucial for purposes of scaling and moving from the laboratory experiment to the industrial scale. According to the numerous data indicating that the optimal conditions for the extraction of phenolic compounds of some plant products are usually different for various plant matrices [4]. Extraction methods that are most commonly used include standard convection methods (maceration, percolation, infusion, decoction, hot continuous extraction) and non-convection methods (ultrasonically extraction (UEA), microwave radiation (MEA), pulse electrical discharge (PAED) and supercritical fluid extraction (SFE) [5]. Specified methods in different options of the experiment were used to conduct numerous studies on the extraction of polyphenols from grape waste for example, including grape seed [6–15].

The purpose of this study was the selection of optimal conditions for effective water solid-liquid extraction (SLE) of polyphenolic compounds from defatted grape seed flour in order to obtain an extract with the maximum antioxidant potential.

Materials and methods

Chemicals

The following chemicals used in this study are as follows: potassium bromide, sodium hydroxide, sodium carbonate (Reachim, Russia); sulphur acid (Sumychemprom, Ukraine); gallic acid (Sigma Aldrich, USA). All the chemicals used in this experiment were of analytical grade. The synthesis of Folin-Ciocalteu reagent was done according to the procedure [16]. All the chemicals used in this procedure were of analytical grade. For analysis 2 M solution was used. For preparation of the solutions distilled water with electric conductivity no more 0.55 mS/m was used. The conductivity was measured by a conductometer CEL-1M2 (Analitpribor, Georgia).

Raw Material

Defatted grape seeds flour (Oleo Vita trademark, Orion, Ukraine) was made from fresh grape seeds of unfermented squeezing in industrial conditions. A mixture of four grape varieties in equal proportions in mass, grown in the southern regions of Ukraine

(Odessa region) was used as the raw material. Gently dried grape seeds at a temperature not more than 60 °C were subject to the thorough cleaning (separation).

Grape seed cake was obtained in the form of solid plates with its further fine grinding and crushing to fine powder (flour) in the process of cold pressing. The residual fat content in the flour was 8-9%.

Extraction Process

Accurately weighted dried powder (according to ratio liquid to solid) were extracted with the solvent by 10 ml in glass test tubes (total volume 15 ml) with screw caps. Distilled water was used as the extractive agent. All the time the tubes were shaken. The extraction was performed in the dark and at corresponding temperature, a solid-liquid ratio and the extraction time. The tubes were placed in a thermostat at a set temperature for the extraction time. The liquid after extraction was separated from solids by centrifugation at 6.0 g for 10 min (OPn-8UHL4.2, Russia).

The solutions were kept at a constant temperature using the thermostat 1TZH-0.03 (Russia). The temperature in this device was maintained at an accuracy of 0.2 °C and determined by the sensor SM60-Pt1000 (Yokogawa Europa, Holland) with a precision of 0.1 °C.

The samples were weighed on laboratory scales balance CBA-300-0.005 (T-Scale, China) with accuracy of 5 mg and on analytical laboratory scales balance VLR-200 (Gosmetr, Russia) with accuracy up to 0.1 mg.

Determination of the Total Antioxidant Capacity

TAC of samples was determined by the reaction with electronegative bromine [17]. In this research using the same method that was detailed [18, 19]. The experimental data of coulometric titration were used to calculate the TAC, as the electricity quantity Q , spent for titration per 100 g of the sample and it were calculated by expression:

$$TAC = \frac{100 Itm_{solution}}{m_{al} m_{sample}}, \quad (1)$$

where m_{sample} is the weight of the sample (dry powder of plant or candy caramel), $m_{solution}$ is the total weight of the solution for candy caramel or of the extract for powder of plant. Values of TAC in gallic acid equivalent (mg GAE/100 g sample) were calculated. For aqueous solutions of gallic acids the coefficients of the linear regression were determined in [18].

Determination of the Total Phenolic Content

The concentration of phenolic compounds in samples was estimated using a modified spectrophotometric Folin-Ciocalteu method according Singleton and Rossi [16] with the transition from volume to weight of the aliquot portion. Briefly, 0.1 g of extract, standard or blank solution was mixed with 0.5 g of Folin-Ciocalteu's reagent and 2.0 g water. A sample of extracts was previously diluted in 10 times. After 8 min, 1.5 g of sodium carbonate 20% (w/w) solution was added to the mixture and adjusted to 10.0 g with distilled water. Mixture was incubated for 30 min in thermostat at 45 °C temperature. Finally, measurement of absorbance was carried out in spectrophotometer SF-46 (Lomo, Russia) and modernized and connected with a computer Specord UV-VIS (JenaAnalytik, Germany) at wavelength of 765 nm against a blank sample.

Gallic acid was used as a standard. The TPC values were expressed as mg of gallic acid equivalents (GAEs) per g of dry weight (DW) of plant. A 1000 mg/kg stock solution of gallic

acid was prepared by dissolving 0.1 g of gallic acid in 100 g of distilled water. Working standard solutions of gallic acid at five different concentration levels (25, 50, 100, 250 and 500 mg/kg) were prepared by dilution of the stock solution. The gallic acid calibration curve was constructed in the range of 25–500 mg/kg and used to calculate linear regression models [18].

The yield (%) of the extraction of phenolic compound (gram to gram dry weight of powder) was defined as :

$$YPC = \frac{TPC}{1000} 100\%, \quad (2)$$

Experimental Design

A response surface methodology (RSM) as a tool for optimization in this research was applied [20]. A tree level, three variable Box–Behnken design [21] was applied to determine the best combinations TAC and TPC (maximum value) of extraction variables for the extraction from defatted grape seeds flour.

Table 1
Independent variables and their levels employed in a Box–Behnken design for optimization of defatted grape seed flour extracts

Independent variables	Symbol	Coded levels		
		-1	0	1
		Natural levels		
Temperature, °C	Temp	60	80	100
Time, min	Time	90	120	150
Ratio volume of liquid to weight of solid powder (v/w), ml/g	R L/S	60	80	100

Three independent variables selected for this study were the extraction temperature (T), the extraction time (t), and ratio volume of liquid to weight of solid powder (R L/S) (Table 1).

Preliminary trials showed as the values of responses changed with increasing temperature, time and ratio L/S; therefore, the optimal levels were selected as center points in the designed experiment. The factorial design consisted of requires an experiment number according to $N=2k(k-1)+cp$, where k is the number of factors (k=3) and (cp) is the number of the central points eight factorial points, and three center points leading to 15 sets of experiments [20].

Regression analysis was performed on the data of dependent variables as effected by the extraction conditions and was fitted into an empiric second order polynomial model as shown in the following equation:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j, \quad (3)$$

where Y is the predicted response; $\beta_0, \beta_i, \beta_{ii}, \beta_{ij}$ are the interception coefficient, the linear terms, the quadratic terms and the interaction terms, respectively; X_i, X_j are the actual levels of the independent variables.

Statistical analysis

Determination of the experimental values were done for a number of parallel measurements (n=4). The Student’s t-test permitted us to check the statistical significance of the regression coefficients. A $p < 0.05$ was considered as statistically significant. The Fisher’s test for analysis of variance (ANOVA) was performed on experimental data to evaluate the

statistical significance of the model. The the Design Expert software trial version 11.0 (Stat-Ease, USA) were employed for the regression analysis and the graphical optimization, respectively.

The models of each response for full-factorial design were expressed in terms of actual variables and without taking into account the statistically insignificant terms.

Results and discussion

Fitting the model of the extraction process from defatted grape seed flour

The experimental data of dependence of TAC and YPC water extract of grape seeds flour from the investigated factors are shown in Table 2.

These experimental data were processed in the framework of analysis of variance (ANOVA) using the model of the average, linear, mixed and quadratic models. As it is commonly known that, ANOVA is a statistical analysis method for assessing the significance of experimental data and for adequacy analysis of the fitting model [20]. Analysis of obtained calculations showed that the most adequate description of TAC and YPC experimental values is achieved in a quadratic model. ANOVA data results for a quadratic model describing the dependence of total antioxidant capacity from the investigated factors are given in Table 3.

Table 2
Experimental of the three-level and three-variable Box–Behnken design and TAC, TPC and yield of polyphenolic compound of defatted grape seed flour extracts

Run	Code pattern			Temp, °C (A)	Time, min (B)	R L/S (C)	Experimental values	
	Temp	Time	R L/S				YPC, %	TAC, GAE mg/g DW
1	0	1	-1	80	150	60	1.625	22.93
2	-1	1	0	60	150	80	1.492	19.62
3	0	-1	-1	80	90	60	1.522	21.89
4	-1	0	-1	60	120	60	1.394	17.71
5	1	0	1	100	120	100	2.550	35.51
6	1	1	0	100	150	80	2.526	36.78
7	0	0	0	80	120	80	1.882	25.98
8	-1	0	1	60	120	100	1.428	20.19
9	0	-1	1	80	90	100	1.757	24.95
10	0	0	0	80	120	80	1.893	26.02
11	0	0	0	80	120	80	1.914	26.02
12	-1	-1	0	60	90	80	1.144	18.14
13	1	-1	0	100	90	80	2.600	35.72
14	1	0	-1	100	120	60	2.288	35.73
15	0	1	1	80	150	100	1.940	27.20

The coefficients R^2 and $R^2_{adjusted}$ are equal to 0.9944 и 0.9843, respectively, according to the results from Table 3. These values indicate that the model adequately describes the experimental data obtained and that they are well coordinated with their predicted values. The model is statistically significant because the p value is less than 0.0001. The obtained value of the variation coefficient (C.V. = 3.19%) is quite low and indicates the reliability of the experimental data.

Table 3
Analysis of variance (ANOVA) of the fitted quadratic polynomial model for the TAC of defatted grape seed flour extracts

Source	Sum of squares	df	Mean square	F-value	p-value	Resume
Model	624.95	9	69.44	98.56	< 0.0001	<i>significant</i>
Residual	3.52	5	0.7045			
Lack of Fit	3.52	3	1.17	1954.98	0.0005	<i>significant</i>
Pure Error	0.0012	2	0.0006			
Cor Total	628.47	14				
Estimated regression coefficients						
Term	Coefficient estimate	df	Std error	F-value	p-value	
A	26.01	1	0.4846	822.55	< 0.0001	
B	8.51	1	0.2968	6.03	0.0575	
C	0.7289	1	0.2968	16.30	0.0099	
AB	1.20	1	0.2968	0.0618	0.8136	
AC	-0.1043	1	0.4197	2.60	0.1677	
BC	-0.6769	1	0.4197	0.5161	0.5047	
A ²	0.3015	1	0.4197	27.76	0.0033	
B ²	2.30	1	0.4368	2.89	0.1500	
C ²	-0.7423	1	0.4368	5.44	0.0669	
Model summary statistics						
Std. Dev.	0.8394	R²			0.9944	
Mean	26.29	Adjusted R²			0.9843	
C.V.%	3.19	Predicted R²			0.9103	
PRESS	56.35	Adeq Precision			28.8892	

The Fisher criterion value of F at 98.56 level implies that the model is statistically significant. The obtained values of p criterion that are less than 0.0500 indicate the significance of the calculated coefficients A, C, A² of equation (3). The calculated F values for Lack of Fit are also statistically significant. The predicted value of Predicted R²=0.9103 is in necessary compliance with Adjusted R² =0.9843, since their difference is less than 0.2. The value of AdeqPrecision, expressing the signal-to-noise ratio, and equal to 28.8892 is greater than the indicative value 4, which indicates data adequacy.

Similar conclusions can be made with respect to the model describing the phenolic compounds output in the extraction process (Table 4). The adequacy of the experimental data description on the phenolic substances output is achieved by indicated model, as evidenced by the high correlation coefficients R² and R²_{adjusted} that are equal to 0.9874 и 0.9646, respectively, and the p value that is equal to 0.0003. The reliability of the experimental data was also confirmed by the value of factor at the level of C.V.=4.59%.

The adequacy of the experimental data description on the phenolic substances output is achieved by indicated model, as evidenced by the high correlation coefficients R² and R²_{adjusted} that are equal to 0.9874 и 0.9646, respectively, and the p value that is equal to 0.0003. The reliability of the experimental data was also confirmed by the value of factor at the level of C.V.=4.59%.

The value of Fisher's criterion F at the level of 43.37 is lower than in the previous review, and it demonstrates better statistical significance. The obtained values of p criterion are less

than 0.0500 and they indicate the significance of the calculated coefficients A, C, A² of equation (3).

The calculated F values for Lack of Fit are also statistically significant. The predicted value of Predicted R² 0.8001 is in reasonable compliance with Adjusted R² 0.9646. The value of Adeq Precision, which represents a signal-to-noise ratio, is 20.6011 greater than the indicative value 4, which indicates data adequacy.

Table 4
Analysis of variance (ANOVA) of the fitted quadratic polynomial model for the YPC of of defatted grape seed flour extracts

Source	Sum of squares	df	Mean square	F-value	p-value	Resume
Model	2.86	9	0.3175	43,37	0,0003	<i>significant</i>
Residual	0.0366	5	0.0073			
Lack of Fit	0.0361	3	0.0120	45,55	0,0216	<i>significant</i>
Pure Error	0.0005	2	0.0003			
Cor Total	2.89	14				
Estimated regression coefficients						
Term	Coefficient estimate	df	Std error	F-value	p-value	
A	1.90	1	0.0494	346,84	< 0.0001	
B	0.5634	1	0.0303	5,35	0,0687	
C	0.0700	1	0.0303	12,21	0,0174	
AB	0.1057	1	0.0303	6,10	0,0566	
AC	-0.1056	1	0.0428	1,76	0,2417	
BC	0.0568	1	0.0428	0,2210	0,6581	
A ²	0.0201	1	0.0428	7,76	0,0386	
B ²	0.1241	1	0.0445	3,22	0,1325	
C ²	-0.0800	1	0.0445	5,62	0,0639	
Model summary statistics						
Std. Dev.	0.0856			R²		0.9874
Mean	1.86			Adjusted R²		0.9646
C.V.%	4.59			Predicted R²		0.8001
PRESS	0.5785			Adeq Precision		20.6011

The Pearson's correlation coefficient for actual and predicted TAC and YPC values at a level of 0.9972 and 0.9936, respectively, indicates the possibility of using the models obtained to predict data on antioxidant capacity and polyphenolic compounds output for defatted grape seed flour when conducting solid-liquid extraction with water.

The following forecast models for describing the properties studied at the code level, which are described by expressions (4) and (5), are subject to proceed to, summarizing all the above:

$$\text{TAC (mg/g DW)} = 26.01 + 8.51A + 0.7289B + 1.20C - 0.1043AB - 0.6769AC + 0.3015BC + 2.30A^2 - 0.7423B^2 - 1.02C^2, \quad (4)$$

$$\text{YPC (mg/g DW)} = 1.90 + 0.5634A + 0.0700B + 0.1057C - 0.1056AB + 0.0568AC + 0.0201BC + 0.1241A^2 - 0.0800B^2 - 0.1056C^2, \quad (5)$$

Effect of process variables on Total Antioxidant Capacity

The effect of independent variables of temperature, extraction time and ratio on the total antioxidant activity is shown in Figure 2. As seen from the scan, TAC value under the effect of independent variables varies within 17.71–36.78 GAE mg/g DW.

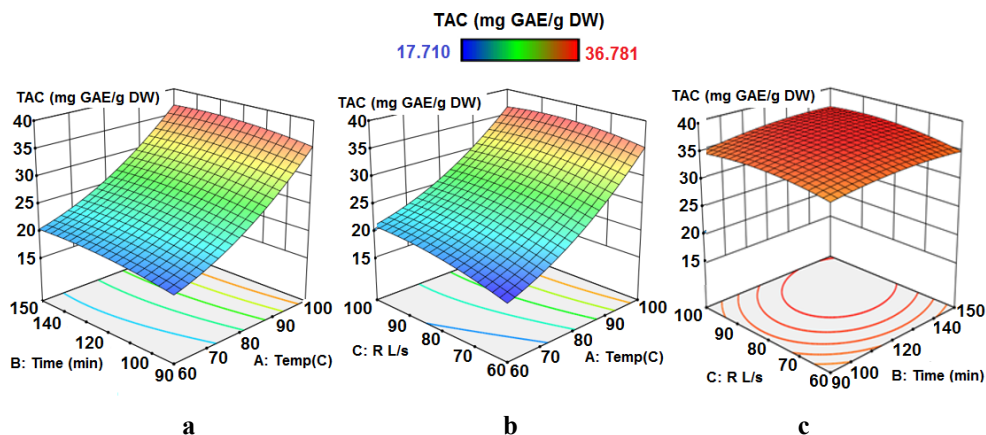


Figure 2. The 3D and 2D response surfaces profiles of TAC as affected by independent variables at optimal conditions: ratio S/L 85 (a), time 131 min (b) and temperature 100 °C (c)

The analysis of three-dimensional surfaces of the basic property dependence on a pair of variables at a fixed optimal value of the third parameter has allowed establishing the following consistent pattern:

- an increase in temperature from 60 to 100 °C leads to steady increase in TAC value regardless of the nature of the second independent parameter and the fixed value of the third parameter (Figure 2, a, b) and the achievement of the maximum TAC=36.78 GAE mg/g DW at a temperature of 100 °C
- when fixing the temperature and considering the dependence of TAC value on extraction time and R L/S, a surface with strongly pronounced single point of extremum, which corresponds to the maximum TAC value at a fixed temperature of 100 °C (Figure 2, c) is obtained.

Effect of process variables on Total Phenolics Content

The effect of temperature independent variables, extraction time and ratio L/S on the phenolic compounds yield is shown in Figure 3. As can be seen from the scan, the output value of phenolic compound varies under the effect of independent variables within 1.14–2.60%. The effect of temperature, as in the case of the total antioxidant capacity, leads to a monotonic increase in the YPC value on the entire studied variation interval from 60 to 100 °C, regardless of the variation of the second parameter (Figure 3, a, b). When the temperature is fixed at the optimal level and the other two parameters variation, the pronounced extreme dependence on the extraction time on all curves, regardless of the value of R L/S (Figure 3, c) is obtained.

The monotonous growth of YPC with an increase in the extraction time without reaching an extremum is observed on projection curves up to R L/S 90 values. The pronounced extreme dependence is weakly detected only after the value of 90.

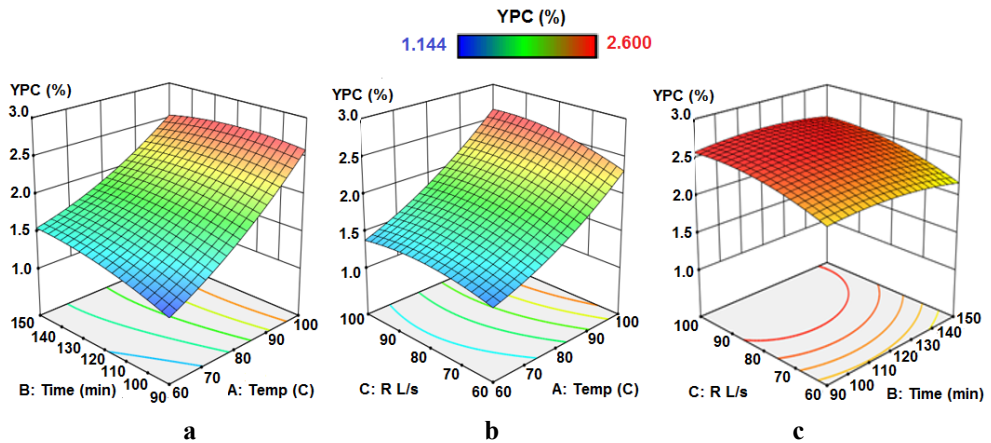


Figure 3. The 3D and 2D response surfaces profiles of YPC as affected by independent variables at optimal conditions: ratio S/L 93 (a), time 117 min (b) and temperature 100 °C (c)

Optimum conditions for SLE from defatted grape seeds flour by water

The regression models obtained from Eqs. 4 and 5 were used to determine optimum values of extraction temperature, time and ratio liquid to solid by using optimization procedures. The objective function (Q) in this optimization was defined to maximize simultaneously the TAC as well as to maximize the yield of phenolic compounds extract with subject to temperature range (A), time (B), and ratio solid to liquid (C):

$$\max Q = \begin{cases} \text{TAC} \\ \text{YPC} \\ \text{TAC} + \text{YPC} \end{cases}, \quad \text{TAC, YPC} = f(A, B, C), \quad -1 < A, B, C < 1. \quad (6)$$

The optimal conditions are fairly simply and efficiently determined using the RSM methodology mentioned above for a single-response function [20]. TAC and YPC 37.04 and 2.646 values were obtained as a result of carrying out the optimization procedure, respectively (Table 4).

It should be noted that the completed optimization procedure using models (equations 4 and 5) makes it possible to obtain more than ten sets of optimal parameters at which the values of the optimal function are in the 1% interval of variation from the maximum value. Although, the differences in the optimal values of the parameters A, B and C are also practically in the same variation interval.

Table 4

Estimated optimum conditions of responses TAC and YPC

	T, °C	t, min	R L/S	TAC, mg GAE/g DW	YPC, %	Desirability
TAC	100	131	85	37.04		0.9585
YPC	100	117	93		2.646	0.9537
TAC+YPC	100	123	89	36.91	2.633	0.9504

It should be noted that the completed optimization procedure using models (equations 4 and 5) makes it possible to obtain more than ten sets of optimal parameters at which the values of the optimal function are in the 1% interval of variation from the maximum value. Although, the differences in the optimal values of the parameters A, B and C are also practically in the same variation interval.

Under multivariate consideration, the Derringer function or the desirability function (Desirability) were used, preferring a set with larger value (Figure 4) as a final selection criterion of the optimal conditions set.

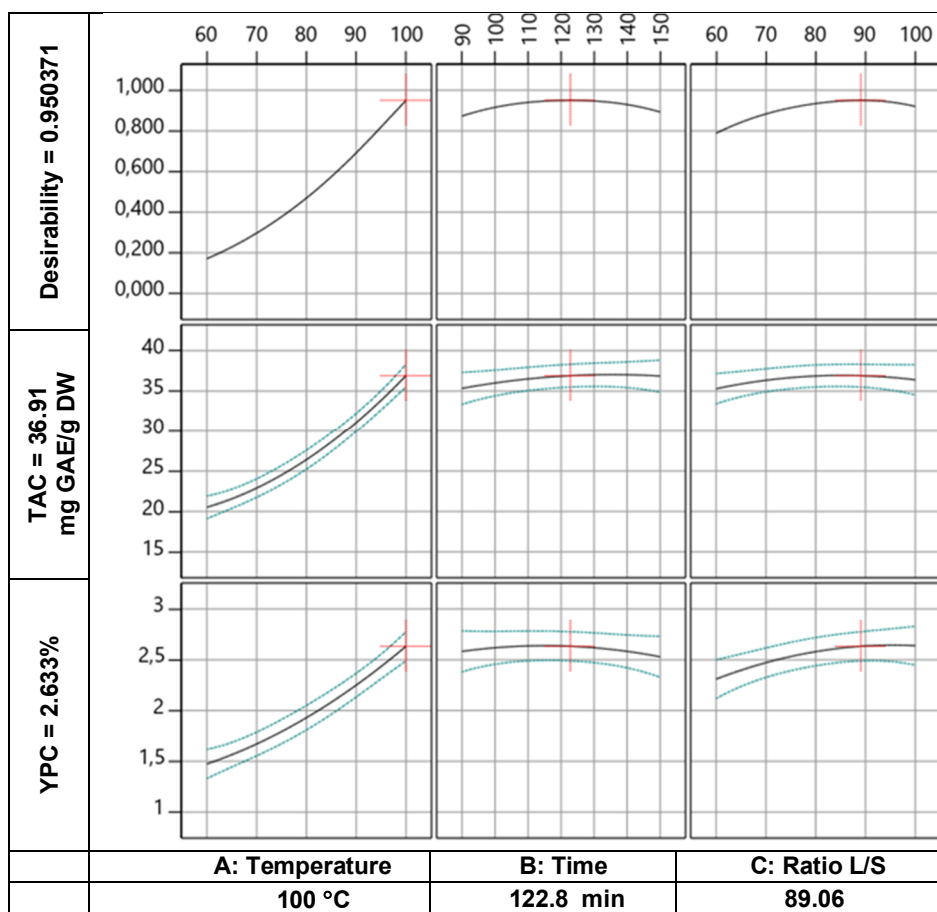


Figure 4. Overall optimum conditions of solid-liquid extraction of phenolic antioxidants from defatted grape seed flour

This function was used as a selection criterion of compromise solution in the multi-criteria optimization technology, in our case with two TAC and YPC response functions. It should be noted that the desirability function is the most important and most frequently used in optimization procedures with multiple response functions [20]. The corresponding optimization algorithm of extraction procedure conditions is effective and economical, when solving the tasks of the output maximization of necessary components from the plant matrix based on several response functions [22].

The results of optimization process of the combination of TAC and YPC values within the variation range of independent extraction variables are presented in Figure 4. The maximum value of the desirability function (0.9504) is reached at a temperature of 100 °C, extraction time of 123 minutes and the ratio of the volume of water to the mass of powder 89 (Table 4). The obtained values of antioxidant capacity and total content of phenolic compounds yield is 36.91mg GAE/g DW and 2.633% less than the similar for each of the properties optimal conditions are only at the level of 0.5%.

Table 5
Comparison between predicted and experimental values in optimal conditions of extractive process (temperature 100 °C, time 123 min, ratio L/S 89)

Value	Predicted	Experimental	δ,%
TAC, mg GAE/g DW	36.91	36.45	1.2
YPC,%	2.633	2.705	2.7

Model validation

The obtained models of TAC and TPC were verified by comparing the predicted data with experimental data. Table 5 shows the comparison between the predicted and experimental values for each response studied at conditions: temperature – 100 °C, time 123 min and ratio L/S 89.

As show Table 5, Experimentally, the values in all three cases are in good agreement with theoretical values within 3%. This can be considered a satisfactory result.

Correlation TAC and TPC values

The various mechanisms of oxidation-reduction reactions, simulating the effect of radical oxidation are used in methods for studying antioxidant properties [23, 24]. The method of galvanostatic coulometry with electrogenerated bromine was used as a method for evaluating antioxidant capacity in this study. The procedure is based on the interaction of antioxidants with bromine and allows evaluating the integral antioxidant capacity of a wide range of materials, including plant materials and materials of a biological nature [25]. The methodology for determining the total content of polyphenols is widely used to estimate the total content of polyphenolic antioxidants, unlike the previous one [24]. Although the different mechanism of reactions effect on which these methodologies are based, there is a quite high positive correlation between the obtained data (Figure 5).

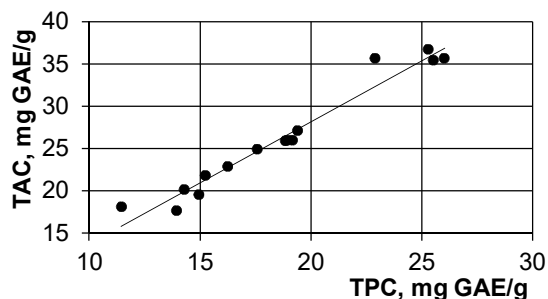


Figure 5. Correlation plots for TAC vs. TPC of water extracts from defatted grape seed flour, $R^2=0.9587$

Similar regularities were obtained for confectionery products with plant extracts [18]. Data concerning the correlation of TAC and TPC values is another proof that the coulometric titration methodology with electrogenerated bromine is sufficiently adequate for the purposes of determining the total antioxidant capacity in extracts and food samples based on them, with the prevailing content of phenolic compounds as antioxidants.

Conclusion

The following conclusions can be drawn, based on the results obtained:

1. As a result of optimization procedure, it has been determined, that upon optimal conditions, in particular the temperature of 100 °C, the extraction time of 131 minutes and the ratio of the extractive agent volume to the mass of powder of 85, the maximum total antioxidant capacity of the extract, that is equal to 37.04 mg GAE/g DW can be obtained. The maximum output of phenolic substances of 2.646% was obtained under the following conditions: the temperature of 100 °C, the extraction time of 117 minutes and the ratio of the extractive agent volume to the mass of powder of 93.
2. The usage of Derringer function as a selection criterion of compromise solution in a multi-criteria optimization technology with two TAC and YPC response functions, makes it possible to quickly and effectively predict the optimal conditions of the extraction procedure: the temperature of 100 °C, the extraction time of 123 minutes and the ratio of the extractive agent volume to the mass of powder of 89 – the conditions under which the values of TAC and YPC 36,91 mg GAE/g DW and 2,633%, were obtained. The validation of obtained results showed their compliance within 3% with experimental values.
3. The high correlation value between the total antioxidant capacity and the total content of phenolic substances indicates that the galvanostatic coulometric titration with electrogenerated bromine is an appropriate procedure for the purpose of quantitative evaluation of total antioxidant potential of the phenolic compounds in plant extracts.

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