EVALUATION OF EXTRACTS FROM DRY FRUITS OF BLACK BLUEBERRY (VACCINIUM MYRTILLUS L.) THROUGH ONE-DIMENSIONAL AND MULTI-DIMENSIONAL REGRESSION ANALYSIS FOR FLAVONOID PHENOLIC COMPOUNDS

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ABSTRACT

The aim of the study is to develop a technology for obtaining extracts of dried fruits of black blueberry. The basic extraction parameters have been established. The influence of the technological parameters of the extraction process on the content of the flavonoidphenoliccompounds in the extracts is analyzed. Mathematical data processing was performed by one-dimensional and multi-dimensional regression analysis. Estimates were made on the degree of influence of the factors as well as on their level of significance. Fischer's criterion is assessed, as well as its probability. Residue assessment and analysis was performed by normal probability plot of residues, the scatter plot of the predicted residual values and the residual histogram. The extracts obtained were determined by the amount of flavonoid phenolic compounds to enrich the fruit juices with BAV. The effect of the extractant type, the duration and temperature of the extraction and the hydromodule on the color parameters were investigated. The results of the planned experiment are statistically processed with the Statistica program. Residue assessment and analysis was performed by normal probability plot of residues, the scatter plot of the predicted residual values and the residual histogram. The results obtained suggest that 70% ethyl alcohol, temperature 65 ° C, duration 3-4 h and 1:30 hydromodule are technologically reasonable choices for obtaining extracts with a maximum content of common flavonoid phenolic compounds. Adequate mathematical models were described describing the dependencies of the individual parameters in the extraction of the common flavonoid phenolic compounds. Technology for obtaining extracts with maximum content of common Adequate mathematical models were described describing the dependencies of the individual parameters in the extraction of the common flavonoid phenolic compounds has been developed.

Keywords: *extracts, black blueberry fruits, flavonoid phenolic compounds, regression analysis.*

I. INTRODUCTION

Studies conducted in different countries confirm that one of the main causes of pathological changes in the human body leading to premature aging and development of cardiovascular diseases,

oncological diseases and diabetes is the excessive accumulation of free radicals and active forms of oxygen in the biological fluid of the organism.
Increasing the content of free radicals in the cells creates conditions for the so- oxidative stress in which free radicals oxidize vessel walls, protein molecules, DNA and lipids.These radicals actively interact with membranes of lipids containing unsaturated bonds and alter the properties of cell membranes [1].
Berries contain powerful antioxidants and a proper balance of bioactive compounds. They are considered to be a good source of phenolic compounds, especially flavonoids and phenolic acids, which mostly contribute to their high antioxidant activity. Berries have recently received much attention for their health benefits, including antimutagenesis and anticarcinogenic activity for the prevention of various cancers and age-related diseases [2].
Beverages are anoptimal form of food that can be used to enrich the nutritional portion of irreplaceable nutrients and biologically active substances that have a beneficial effect on metabolism and immune resistance of the body [3].
In order to increase the nutritional value and antioxidant properties of juice-containing beverages, extracts of wild-growing raw materials having a prophylactic and functional effect can be introduced into the production technologies.
The aim of the study is to develop a technology for obtaining extracts of dried fruits of black bilberries. The basic extraction parameters have been established. The resulting extracts are analyzed for the purpose of enriching fruit juices with flavonoid phenolic compounds.

II. MATERIAL AND METHODS

A. Material. The object of the study is the fruits of *Vaccinium myrtillus* L. In wild plants, there are a number of BAV that can affect the life processes of the human body.
Forest fruits are rich in phenolic compounds and have great antioxidant activity. This makes them a potential raw material for producing extracts that can be used to develop functional beverages.Various variants of water and ethanol extracts from dried berries and black currant have been developed. The aqueous and ethanol extracts of the fruits are respectively 1:10, 1:20 and 1:30 fruit / extract ant hydromodul; at an extraction temperature of 35° -80° C and 1, 2, 3 and 4 hours.The physicochemical analyzes were conducted using standardized methods approved by good manufacturing practice.For each of the test quantities the mean values of three independent experiments are presented. Flavonoid phenolic compounds - spectrophotometric as a rutin,% by pharmacopoeial method [Pharmacopeia Russia]. General method of analysis [4].

B. Mathematical methods. Mathematical data processing was performed by one-dimensional and multi-dimensional regression analysis. By which were studied and evaluated the possible functional dependencies between two or more random variables.The main questions are whether there is a functional dependence between two dependent random variables and if so - to find a function that describes it sufficiently accurately. Various models have been studied, with the best-described dependencies being selected. Estimates were made on the degree of influence of the factors as well as on their level of significance. Fischer's criterion is assessed, as well as its probability.Residue assessment and analysis was performed by normal probability plot of residues, the scatter plot of the predicted residual values and the residual histogram. All results are presented analytically and graphically.
The processing was done through the statistical program STATISTICA (Stat Soft, Inc.).
All data are processed at level of significance α=0,05.

III. RESULTS AND DISCUSSION

The experimental results obtained for the effect of the type and concentration of the extractant were used to obtain a regression model as well as to study its suitability. After studying the polynomials of the first and second degrees, a model of the following appears to be the best:

$$y = b_0 + b_1 x + b_2 x^2 \quad (1)$$

where x is the percentage of ethyl alcohol as a percentage and y is the concentration of flavonoid phenolic compounds, mg%.

After statistical processing of the data, it is evident that the coefficient of determination $R^2 = 0,999$, which means that 99% of the change in parameter Y is due to the control factor x and is described with the model used. Of all the models studied, the coefficient of certainty is the highest. All the coefficients of the model are statistically significant, since $p - level << 0,05$ they are as follows:

$$b_0 = 59,95932 \; , \; b_1 = 2,16229 \; b_2 = -0,01665 \quad (2)$$

Fisher's criterion, F (2,9) = 9886,7, and its corresponding probability indicate that the model describes a significant part of the change in Y. The model performs better than the so-called naive average forecasts.

The regression equation is:

$$y = 59,95932 + 2,16229\,x - 0,01665\,x^2 \quad (3)$$

The resulting regression model describes the rights $y = f(x)$, that we can depict in R^2 .

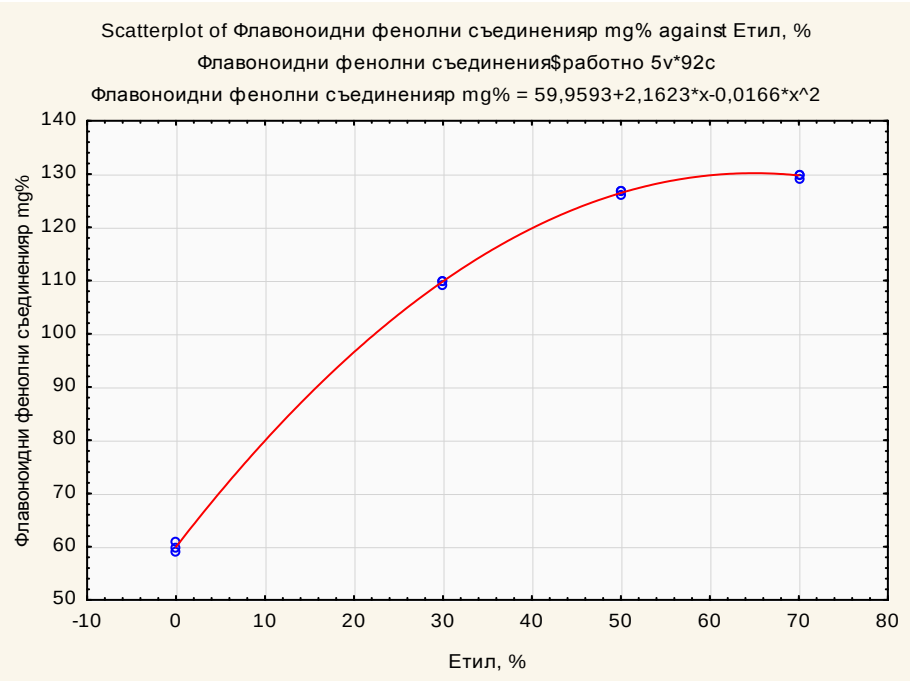

Fig.1. Model response line

The analysis of residues and their graphical representations are shown in Figure 2 in the so-called normal probability graph.

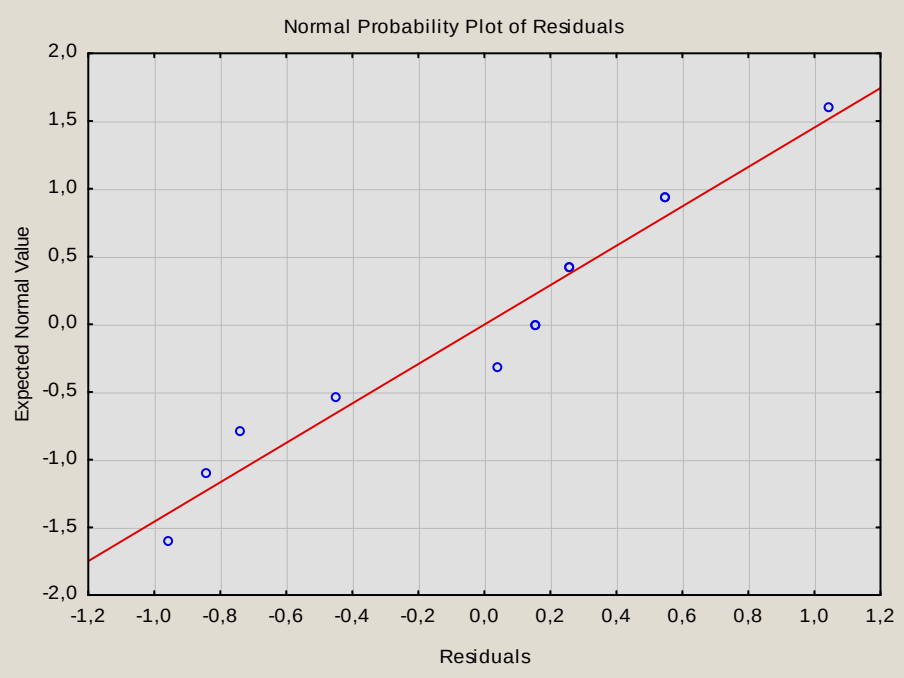

Fig.2. Normal probability plot of residuals

The analysis shows a lack of systematic deviation of the actual data from the theoretical curve, which indicates a normal distribution of residues.

We will check for residual dependence on predicted values from the model. For this purpose, we will analyze the scatterplot of the residuals from the predicted values - FIG. 3.

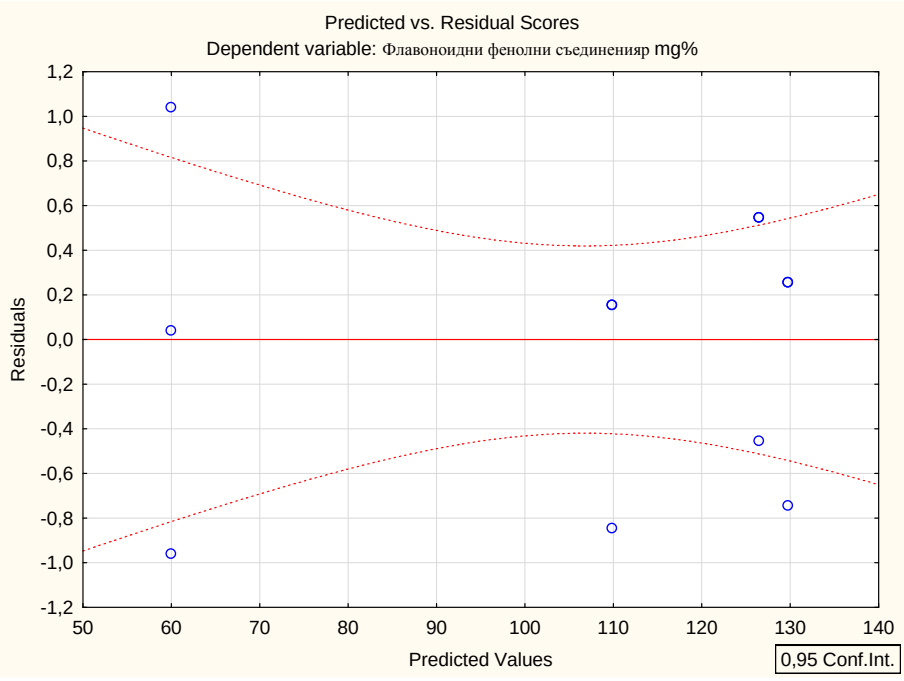

Fig. 3. Scatterplot of residual values from predicted values

The obtained graph shows that the systematic residuals are lacking and are sufficiently chaotic. We can conclude that the residuals do not depend on the predicted values.

To Conclude. From the obtained results we can draw the following conclusions:

1. From the analysis of residuals we can conclude that the obtained model is adequate.
2. The resulting model is linear and describes with great precision the experimental data obtained.

The experimental results obtained for the effect of the extraction temperature were used to obtain a regression model as well as to study its suitability. After studying the polynomials of the first and second degrees, a model of the following appears to be the best:

$$y = b_0 + b_1 x \quad (4)$$

Where **x** is the temperature in degrees Celsius and **y** is the concentration of flavonoid phenolic compounds, mg%.

After the statistical processing of the data it can be seen that the coefficient of determination $R^2 = 0,86$, which means that 86% of the change in parameter **Y** is due to the control factor x and is described with the model used. Of all the models studied, the coefficient of certainty is the highest. All the coefficients of the model are statistically significant, since $p - level << 0,05$ they are as follows:

$$b_0 = 70,10556,\ b_1 = 0,61889 \quad (5)$$

Fischer's criterion, **F** (1,10) = 56,145 p <, 00002, as well as its corresponding probability, show that the model describes a significant part of the change in **Y**. The model performs better than the so-called naive estimates of the averages .

The regression equation is:

$$y = 70,10556 + 0,61889\,x \quad (6)$$

The resulting regression model describes the rights $y = f(x)$, that we can depict in R^2 .

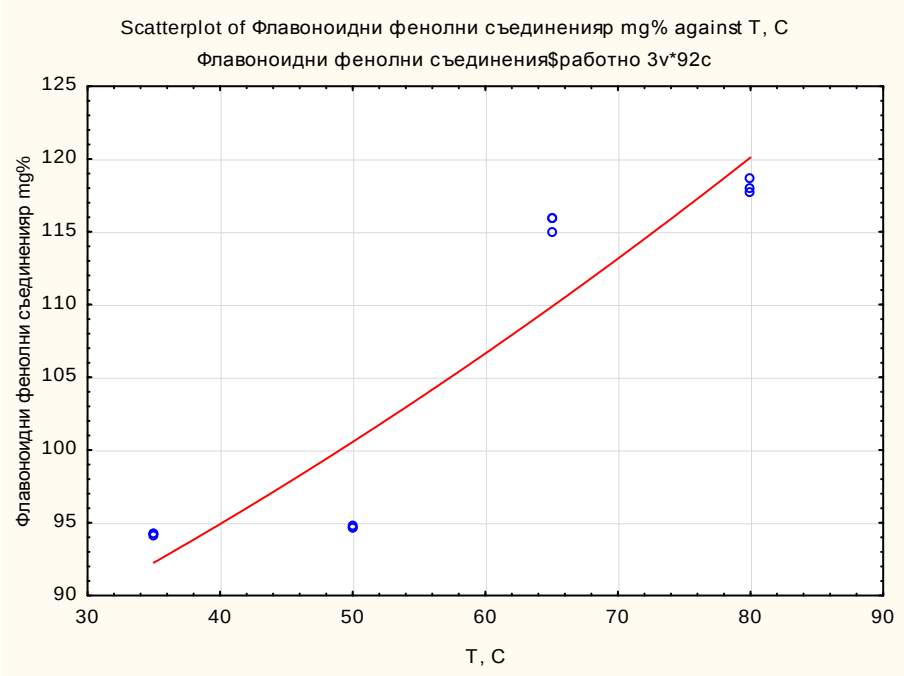

Fig.4. Model response line

The analysis of residues and their graphical representations are shown in Figure 5 in the so-called normal probability graph.

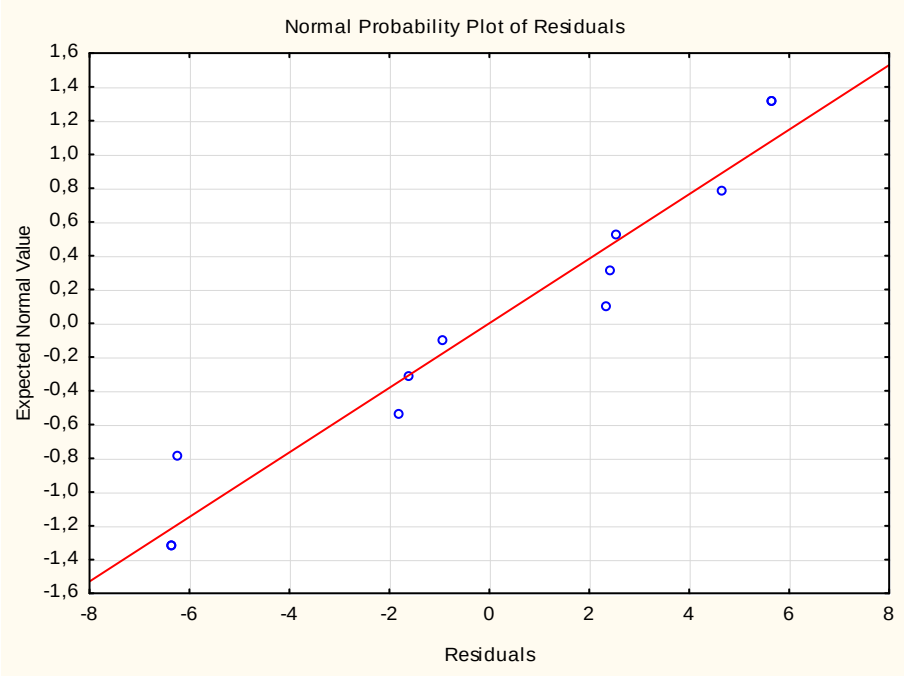

Fig.5. Normal probability plot of residuals

The analysis shows a lack of systematic deviation of the actual data from the theoretical curve, which indicates a normal distribution of residues.

We will check for residual dependence on predicted values from the model. For this purpose, we will analyze the scatterplot of the residuals from the predicted values - FIG. 6.

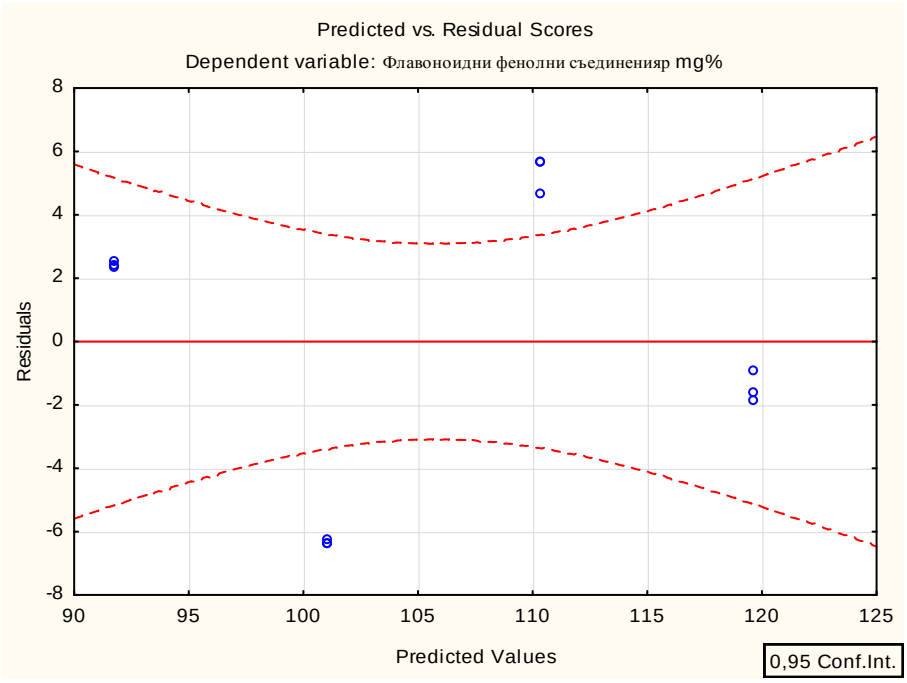

Fig. 6. Scatterplot of residual values from predicted values

The obtained graph shows that the systematic residuals are lacking and are sufficiently chaotic. We can conclude that the residuals do not depend on the predicted values.

To conclude. From the obtained results we can draw the following conclusions:

3. From the residue analysis, we can conclude that the model obtained is adequate.
4. The resulting model is linear and describes with great precision the experimental data obtained.

The experimental results obtained for the effect of extraction time were used to obtain a regression model as well as to study its suitability. After studying the polynomials of the first and second degrees, a model of the following appears to be the best:

$$y = b_0 + b_1 x \quad (7)$$

Where **x** is the time in minutes and **y** is the concentration of Flavonoid phenolic compounds, mg%.
After the statistical processing of the data it is seen that the coefficient of determination $R^2 = 0,98$, which means that 98% of the change in parameter **Y** is due to the control factor x and is described with the model used. Of all the models studied, the coefficient of certainty is the highest. All the coefficients of the model are statistically significant, since $p - level << 0,05$ they are as follows:

$$b_0 = 103,8333, \quad b_1 = 0,1978$$

Fisher's criterion, **F** (2,9) = 9886,7 F (1,10) = 433,43 p <, 00000, and its corresponding probability indicate that the model describes a significant part of the change in **Y**. The model performs better than the so-called naive average forecasts.
The regression equation is:

$$y = 103,8333 + 0,1978\,x \quad (8)$$

The resulting regression model describes the rights $y = f(x)$, that we can depict in R^2.

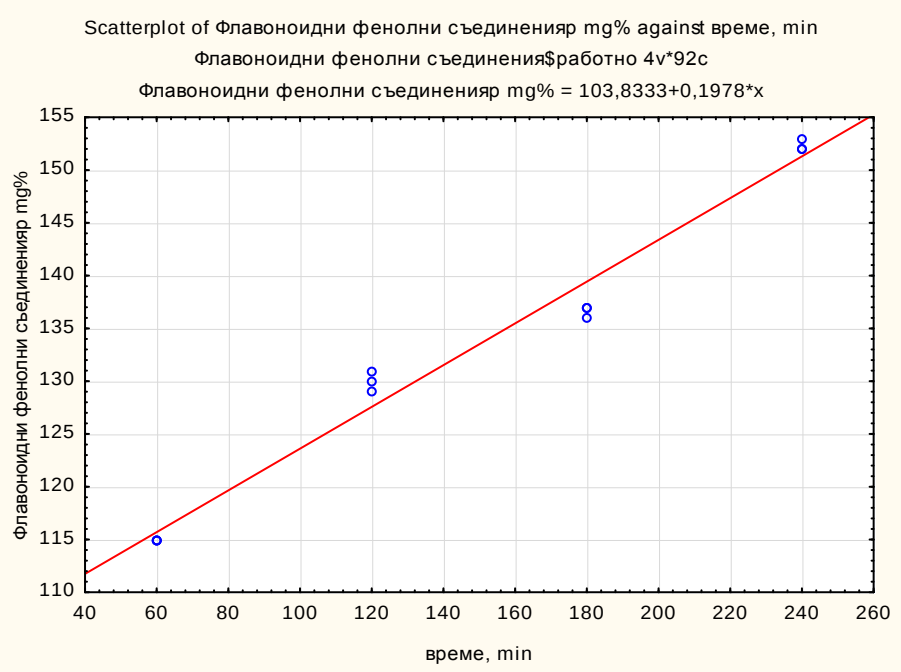

Fig.7. Model response line

The analysis of the residuals and their graphical representations are shown in Fig. 8 in the so-called normal probability graph.

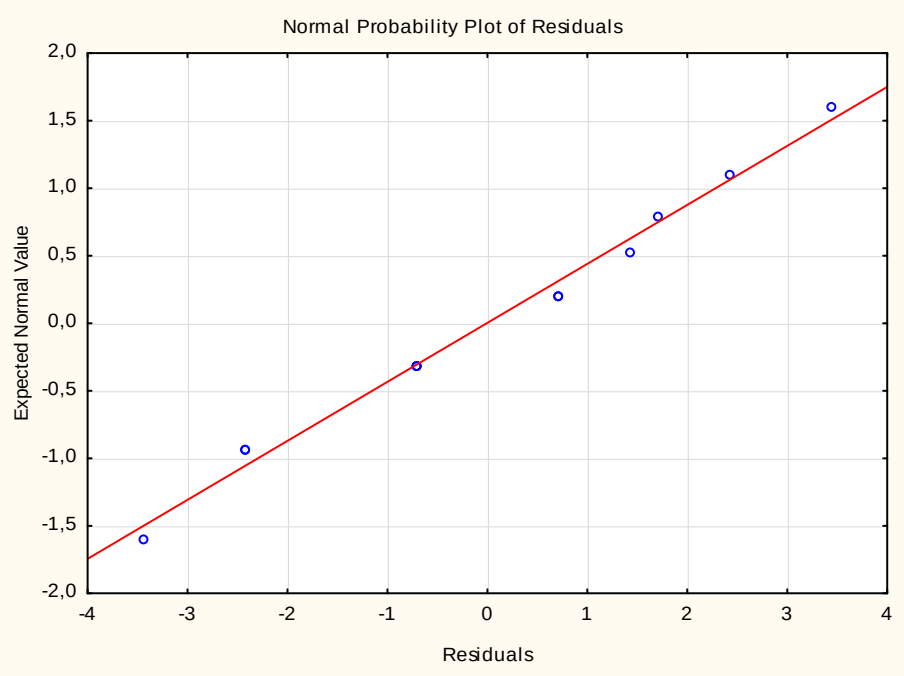

Fig.8. Normal probability plot of residuals

The analysis shows a lack of systematic deviation of the actual data from the theoretical curve, which indicates a normal distribution of residues.

We will check for residual dependence on predicted values from the model. For this purpose, we will analyze the scatterplot of the residuals from the predicted values - FIG. 9.

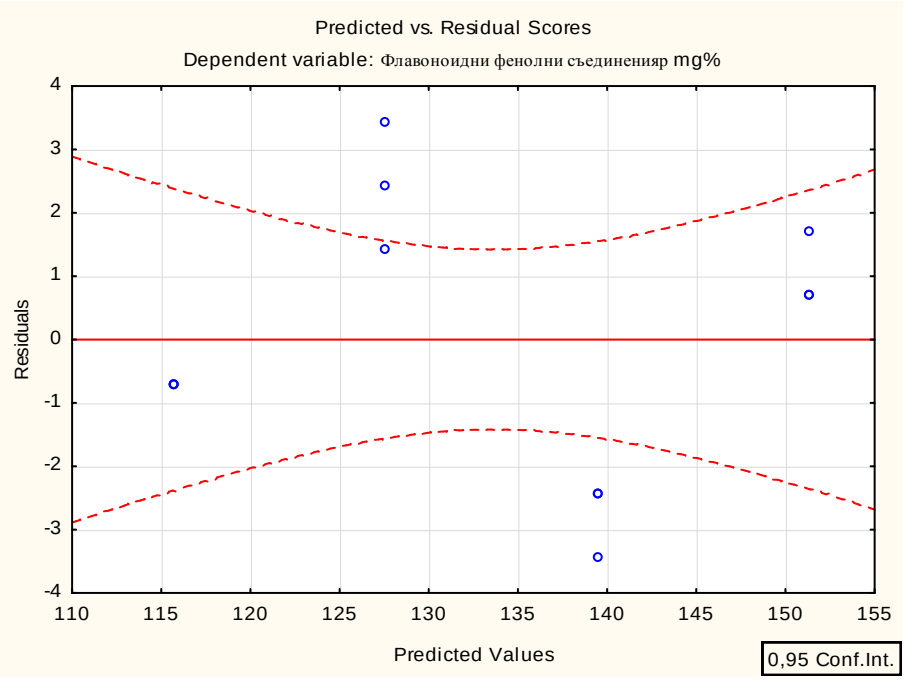

Fig.9. Scatterplot of residual values from predicted values

The obtained graph shows that the systematic residuals are lacking and are sufficiently chaotic. We can conclude that the residuals do not depend on the predicted values.

To conclude. From the obtained results we can draw the following conclusions:

5. From the analysis of residuals we can conclude that the model obtained is adequate.
6. The resulting model is linear and describes with great precision the experimental data obtained.

The experimental results obtained for the influence of the extraction hydromodule were used to obtain a regression model as well as to study its suitability. After studying the polynomials of the first and second degrees, a model of the following appears to be the best:

$$y = b_0 + b_1 x + b_2 x^2 \quad (9)$$

Where **x** is the hydromodule and **y** is the concentration of Flavonoid phenolic compounds, mg%.
After statistical processing of the data, it is evident that the coefficient of determination $R^2 = 0,999$, which means that 99% of the change in parameter **Y** is due to the control factor x and is described with the model used. Of all the models studied, the coefficient of certainty is the highest. All the coefficients of the model are statistically significant, since $p - level << 0,05$ they are as follows:

$$b_0 = 73,00000 \text{ , } b_1 = 7,48333 \; b_2 = -0,11500$$

Fisher's criterion, **F** (2,6) = 4727.4 p <, 00000, and its corresponding probability indicate that the model describes a significant part of the change in **Y**. The model performs better than the so-called naive forecasts average values.
The regression equation is:

$$y = 73,00000 + 7,48333\,x - 0,11500\,x^2 \quad (10)$$

The resulting regression model $y = f(x)$, describes the rights that we can depict in R^2 .

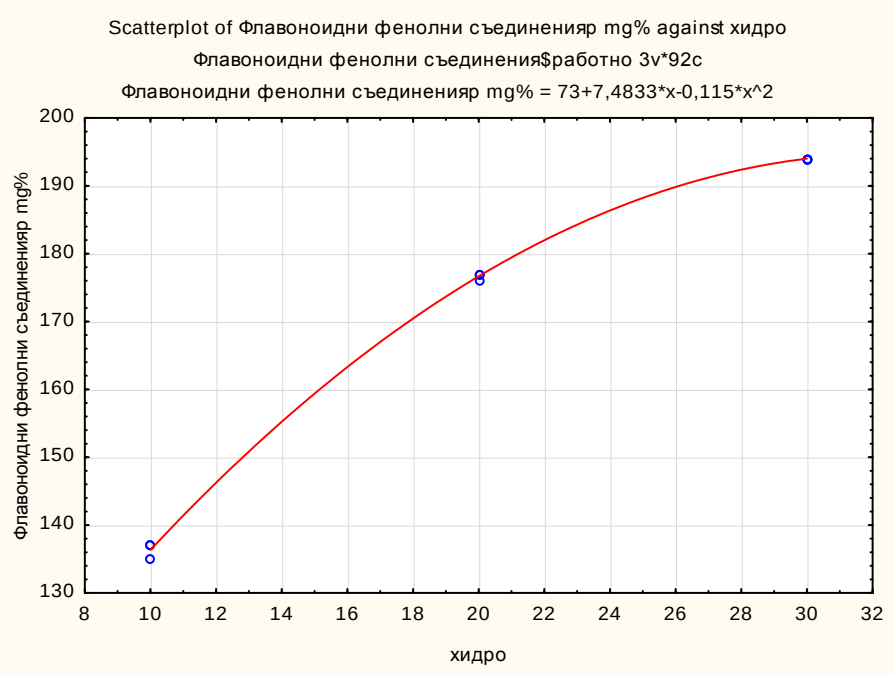

Fig.10. Model response line

The analysis of residuals and their graphical representations are shown in Figure 11 in the so-called normal probability graph.

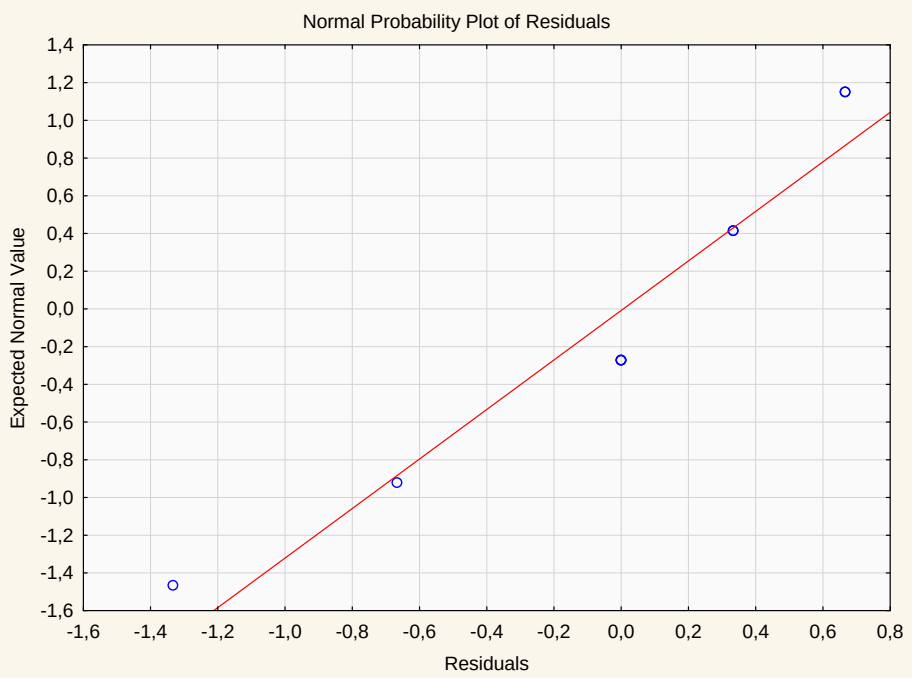

Fig.11. Normal probability plot of residuals

The analysis shows a lack of systematic deviation of the actual data from the theoretical curve, which indicates a normal distribution of residues.

We will check for residual dependence on predicted values from the model. For this purpose, we will analyze the scatterplot of the residuals from the predicted values - FIG. 12.

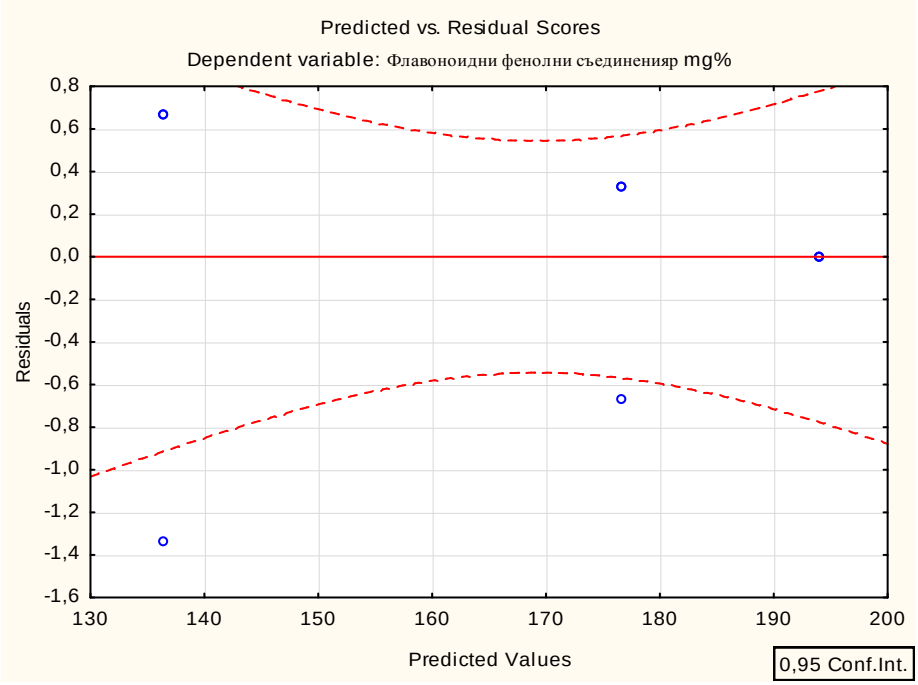

Fig.12. Scatterplot of residual values from predicted values

The obtained graph shows that the systematic residuals are lacking and are sufficiently chaotic. We can conclude that the residuals do not depend on the predicted values.

To conclude. From the obtained results we can draw the following conclusions:

7. From the analysis of residuals we can conclude that the model obtained is adequate.
8. The resulting model is linear and describes with great precision the experimental data obtained.

The experimental results obtained for the effect of ethyl alcohol concentration and extraction temperature on the concentration of flavonoid phenolic compounds were used to obtain a regression model as well as to study its suitability. We will look for multiple regressions between flavonoid phenolic compounds mg% - as a function of response and concentration of ethyl alcohol in percent and temperature in degrees Celsius. The best model turns out to be:

$$z = b_0 + b_1 x + b_2 T + b_3 x^2 + b_4 T^2$$

(11)

Where **x** is the percentage of ethyl alcohol in percent, T is the temperature and **z** is the concentration of Flavonoid phenolic compounds in mg%.

After the statistical processing of the data, it can be seen that the coefficient of determination $R^2 = 0,92$, which means that 92% of the change in parameter **Z** is due to the control factors **x** and **T** is described with the model used. Of all the models studied, the coefficient of certainty is the highest. The statistically significant coefficients of the model are as follows:

$$b_0 = 109,4582 \; b_1 = 2,2710 \; b_2 = -3,1015 \; b_3 = -0,0191$$

$$b_4 = 0,0309$$

Fisher's criterion, F (4,19) = 51,097 p <, 00000, as well as its corresponding probability indicate that the model describes a significant part of the change in **Z**. The model performs better than the so-called naive estimates of the averages.

The regression equation is:

$$z = 109,4582 + 2,2710\,x - 3,1015\,t - 0,0191\,x^2 + 0,0309\,T^2$$

(12)

The resulting regression model $z = f(x, y)$ describes the surface that we can depict in R^3.

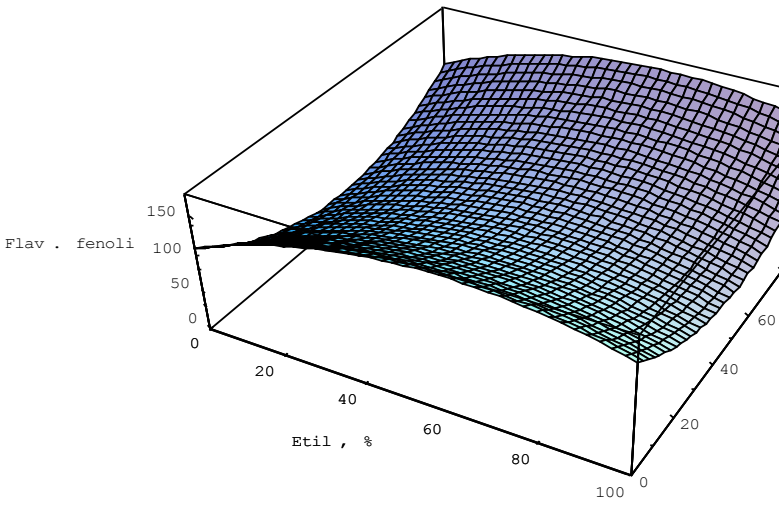

Fig.13. Model response line

The analysis of residuals and their graphical representations are depicted in Fig. 14 in the so-called normal probability graph.

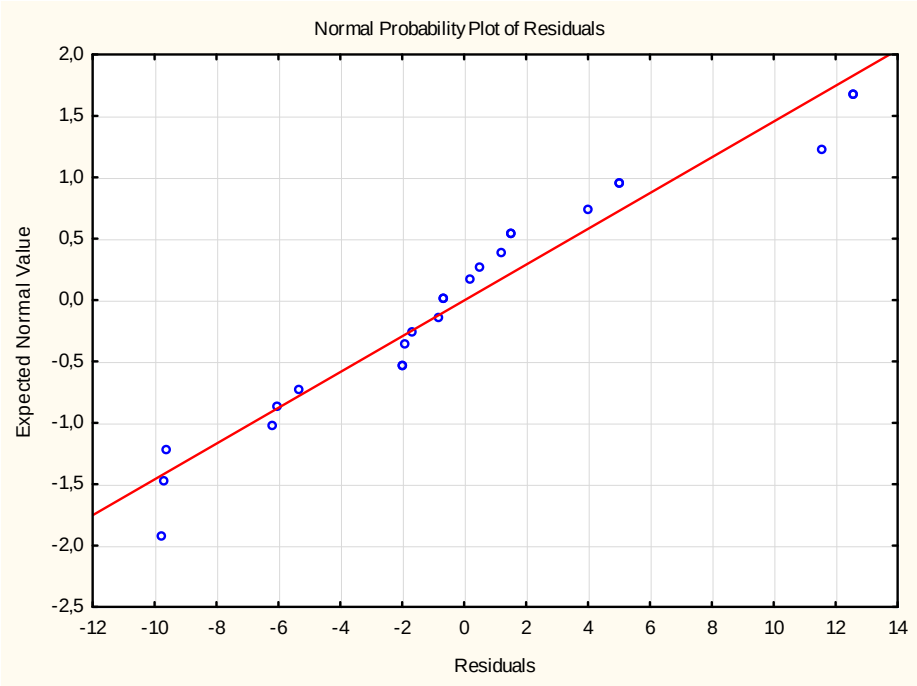

Fig.14. Normal probability plot of residuals

We will check for residual dependence on predicted values from the model. For this purpose, we will analyze the scatterplot of the residuals from the predicted values - FIG. 15.

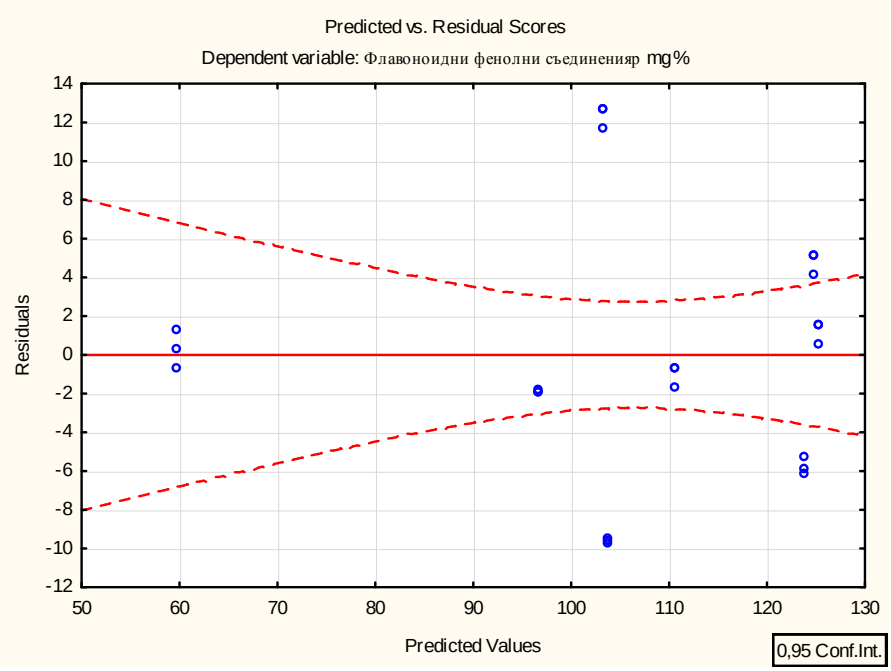

Fig.15. Scatterplot of residual values from predicted values.

The obtained graph shows that the systematic residuals are lacking and are sufficiently chaotic. We can conclude that the residuals do not depend on the predicted values.

To conclude. From the obtained results we can draw the following conclusions:

9. The resulting model is quadratic and describes with great precision the experimental data obtained.
10. From the residue analysis, we can conclude that our model is adequate.
11. From the standardized coefficients, it can be seen that the two factors - temperature and concentration have approximately the same effect, with a slight preference for the temperature by about 20%.

The experimental results obtained for the effect of the concentration of ethyl alcohol and the duration of extraction on the concentration of flavonoid phenolic compounds were used to obtain a regression model as well as to study its suitability. We will look for multiple regressions between flavonoid phenolic compounds mg% as a function of response and ethyl alcohol concentration in percent and time in minutes. The best model turns out to be:

$$z = b_0 + b_1 x + b_2 t + b_3 x^2 + b_4 t^2 \quad (13)$$

Where **x** is the percentage of ethyl alcohol in percent, t is the time in minutes, and **z** is the concentration of Flavonoid phenolic compounds in mg%.

After the statistical processing of the data it can be seen that the coefficient of determination $R^2 = 0,96$, which means that 96% of the change in parameter **Z** is due to the control factors **x** and **t** is described with the model used. Of all the models studied, the coefficient of certainty is the highest. The statistically significant coefficients of the model are as follows:

$$b_0 = 23,03852 \; b_1 = 2,16249 \; b_2 = 0,29188 \; b_3 = -0,01665$$

$$b_4 = -0,00018$$

Fisher's criterion, **F** (4,22) = 123,61 p <, 00000, and its corresponding probability indicate that the model describes a significant part of the change in **Z**. The model performs better than the so-called naive forecasts average values.

The regression equation is:

$$z = 23,03852 + 2,16249\,x + 0,29188\,t - 0,01665\,x^2 - 0,00018\,t^2 \quad (14)$$

The resulting regression model $z = f(x, y)$, describes the surface that we can depict in R^3.

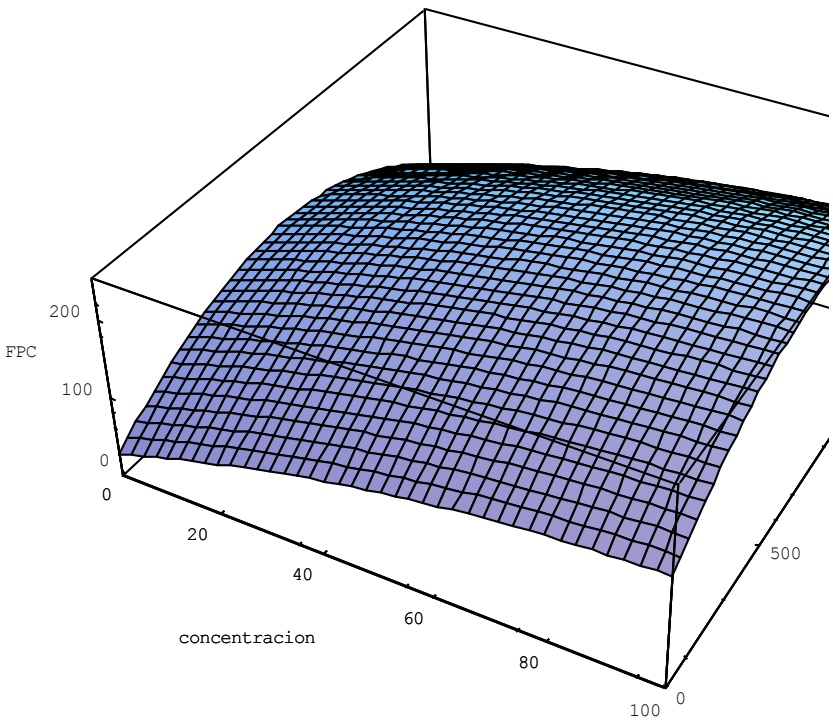

Fig.16. Model response line

The analysis of the residuals and their graphical representations are shown in Figure 17 in the so-called normal probability graph.

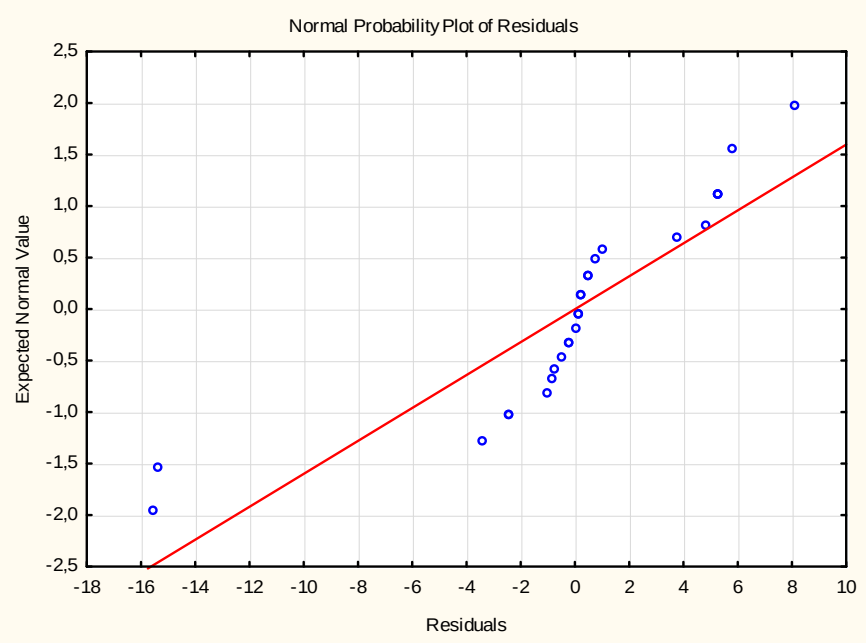

Fig. 17. Normal probability plot of residuals

We will check for residual dependence on predicted values from the model. For this purpose, we will analyze the scatterplot of the residuals from the predicted values - FIG. 18.

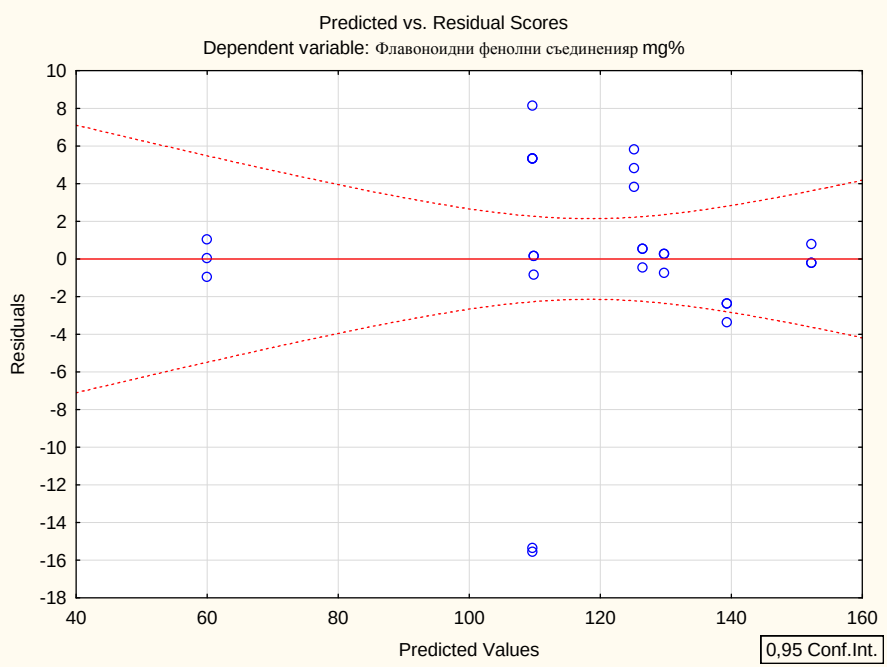

Fig.18. Scatterplot of residual values from predicted values

The obtained graph shows that the systematic residuals are lacking and are sufficiently chaotic. We can conclude that the residuals do not depend on the predicted values.

To conclude. From the obtained results we can draw the following conclusions:

12. The resulting model is quadratic and describes with great precision the experimental data obtained.
13. From the residue analysis, we can conclude that our model is adequate.

14. From the standardized coefficients it can be seen that the time factor exerts a threefold increase in the response function of the concentration.

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