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Assessment of extracts from dry fruits of *Vaccinium myrtillus* L. and *Ribes nigrum* by regression analysis

Antoaneta Georgieva, Radostina Stefanova and Krasimir Krastev

Trakia University, Faculty of Techniques and Technologies, 8602 Yambol, Bulgaria
E-mail: ageorgieva@ftt.uni-sz.bg; r_yorgovi@yahoo.com; krasimir.krastev@trakia-uni.bg

Abstract

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The aim of the study is to develop a technology for obtaining extracts of dried fruits of currants and bilberries. The basic extraction parameters have been established. The influence of the technological parameters of the extraction process on the content of the total phenol compounds 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 resulting extracts determine the amount of total phenolic compounds for the purpose of enriching 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 phenolic compounds. Adequate mathematical models were described describing the dependencies of the individual parameters in the extraction of the common phenols. Technology for obtaining extracts with maximum content of common phenols has been developed.

Keywords: extracts; blackcurrant berries; bilberry fruits; phenolic compounds; regression analysis

Introduction

The production of healthy and complete food is an important and priority task related to the development and implementation of functional food products.

One of the conditions for creating a functional product is to reach the maximum possible level of its nutritional and biological value and guaranteed safety. 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 (Yashin, 2009).

Blueberries and black currant extracts serve as natural antioxidants. 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 (Vesna et al., 2010). As an alternative to the synthetic antioxidants, natural polyphenols from various plant species may be used. These compounds have an ideal chemical structure to “scavenge” free radicals, demonstrating, at the same time, higher antioxidant capacity (e.g., cyanidin and malvidin) than vitamins C and E (Rice-Evans et al., 1996). Blackcurrant (*Ribes nigrum* L.) is a shrub commonly grown invariousparts of theworldoftemperate climate.It tasteful fruits are a rich source of vitamin C and other health beneficial substances such as: routine, organic acids, pectins, micro-and macro nutrients and essential oils (Mattila et al., 2011). Blackcurrant fruits contain polyphenolic substances with antioxidant, antimicrobial, antiviral, and antibacterial properties (Krisch et al., 2009; Molan & Kruger, 2010; Tabart et al., 2012; Brangoulo & Molan, 2011; Petelska et al., 2012).

Contain quercetin derivatives, as indicated in many studies have antimicrobial, anti-inflammatory, antiviral, antitoxic, antiseptic, and antioxidant effects, and are supposed to support the treatment of cancers (Movileanu et al., 2000; Benzie, 2003; Hou et al., 2004). The extracts of blueberries *Vaccinium myrtillus* (bilberry) and Ribes *nigrum* have been analyzed by highperformance liquid chromatography (HPLC) coupled with photodiode array detection and electrospray ionization – mass spectrometry (LC/PDA/ESI-MS).

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 currants and bilberries. The basic extraction parameters have been established. The resulting extracts are analyzed for the purpose of enriching fruit juices with phenolic compounds.

Material and Methods

The object of the study is the fruits of *Vaccinium myrtillus* L. and *Ribes nigrum*. In fruits 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.

Developed various variants of water and ethanol extracts from dried berries blackcurrant have been. The aqueous and ethanol extracts of the fruits are respectively at hydromodul 1:10, 1:20 and 1:30 – fruit/extract; 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. We use Total Phenolic Compounds (AFP) spectrophotometric method with Folin – Denisa Reagent, % as gallic acid at maximum absorption at a wavelength of 765 nm in a cuvette with a layer thickness of 10 mm (General Methods of Analysis, 1987).

For each of the test quantities the mean values of three independent experiments are presented.

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 (StatSoft, Inc.). All data are processed at level of significance $\alpha = 0.05$.

Results and Discussion

The content of the total phenolic compounds in the water and ethanol extracts of the dry berries black currant depends on the correct choice of the process scheme for obtaining. The application of mathematical and statistical methods makes it possible to evaluate objectively the studied variants. In the preparation of extracts of dried berries black currant, influence on the physicochemical properties has the technology of drying the raw material. Proper drying increases the shelf life and increases of the phenolics complex content. On the basis of experimental data, a technological regime for the drying of the studied berries was chosen: convective drying at 50–52°C for 24 hours. The fruit is ground to a particle size of less than 1 mm. Dried fruits can be stored for 2 years. To determine the optimal solvent for the preparation of the extracts: water, a mixture of water and ethyl alcohol in the ratios: 100% H_2O, 30% C_2H_5OH, 50% C_2H_5OH, 70% C_2H_5OH.The use of 70% C_2H_5OH as an extractant is technologically justified to ob-

Table 1. Extracts of the dried fruits of *Vaccinium myrtillus* L.

Model	Regression model	Coefficient of determination R^2
hydromodule 1:10 T = 20°C, t = 24 h, Ethanol – 0, 30, 50, 70%	$y = 2.513676 + 0.0468x$	0.991
hydromodule 1:10, T = 35, 50.65, 80°C, t = 1 h, Ethanol – 70%	$y = 216.0707 – 0.60149x + 0.00742x^2 – 0.00002x^3$	0.999
hydromodule 1:10, T = 65°C, t = 1, 2, 3, 4 h, Ethanol – 70%	$y = 4.175833 + 0.016653x – 0.000043x^2$	0.963
hydromodule 1:10, 1:20, 1:30 T = 65°C, t = 3 h, Ethanol – 70%	$y = 6.093333 – 0.056333x + 0.0003267x^2$	0.999

Table 2. Extracts of dried fruits of *Ribes nigrum*

Model	Regression model	Coefficient of determination R^2
hydromodule 1:10, T = 20°C, t = 24 h, Ethanol – 0.30.50, 70%	$y = 0.219042 + 0.015122x – 0.0001x^2$	0.996
hydromodul 1:10, T = 35, 50.65, 80°C, t = 1 h, Ethanol – 70%	$y = –0.583426 + 0.037981T – 0.000293T^2$	0.837
hydromodule 1:10, T = 65°C, t = 1, 2, 3, 4 h, Ethanol – 70%	$y = 0.780833 – 0.001725x + 0.00001x^2$	0.933
hydromodule 1:10, 1:20, 1:30 T = 65° C, t = 3 h, Ethanol – 70%	$y = 0.634222 + 0.0131x$	0.992

Table 3. Extracts of the dried fruits of *Vaccinium myrtillus* L.

Model	Regression model	Coefficient of determination R^2
hydromodule 1:10, 1:20, 1:30 T = 20°C, t = 1 h, Ethanol – 0, 30, 50, 70%	$z = 2.697706 + 0.114609x + 0.018965y$	0.877
hydromodule 1:10, T = 20°C, t = 1, 2, 3, 4 h, Ethanol – 0, 30, 50, 70%	$z = 0.0083x + 0.001259t – 0.000001t^2$	0.753
hydromodule 1:10, t = 1 h, T = 35, 50.65, 80°C, Ethanol – 0, 30, 50, 70%	$z = 623.3413 + 9.6575x – 27.0228T – 0.1025x^2 + 0.2233T^2$	0.992

tain extracts with a maximum content of common phenols (Figures 1, 5). In order to determine the optimum extraction temperature, four temperature variants were studied: 35° C, 50°C, 65°C and 80°C, and 70% C_2H_5OH as extractant.

The results obtained show that the total extraction temperature for dry fruits of blackberry and blackcurrant is 65°C (5.05 g/0.70 g / 100 g). (Figures 2, 6). To determine the process life extraction was performed at the optimal solvent con and optimal extraction temperature. The optimal extraction time is established – 3–4 hours. During this extraction period a maximum content of the total phenolic compounds is obtained (Figures 3, 7). The effect of the extraction hydromodule on the content of the total phenolic compounds in the extracts from the dried fruits of blackberry and black currant is investigated. It is evident from Figures 4, 8 and 9 that the most suitable hydromodule for extraction is 1:30 (Tables 1, 2, 3 and 4).

From the analysis of standardized coefficients β, it becomes clear that the temperature has a greater impact on the response (Figure 10).

Table 4. Extracts of dried fruits of *Ribes nigrum*

Model	Regression model	Coefficient of determination R^2
hydromodule 1:10, T = 35, 50, 65, 80°C, t = 1, 2, 3, 4 h, Ethanol – 70%	$z = 0.001866x + 0.041784y - 0.000001x^2 - 0.000324y^2$	0.66
hydromodule 1:10, T = 20°C, t = 1, 2, 3, 4 h, Ethanol – 0, 30, 50, 70%	$z = 0.015127x + 0.002661y - 0.0001x^2 - 0.000002y^2$	0.69
hydromodule 1:10, 1:20, 1:30 T = 20°C, t = 1 h, Ethanol – 0, 30, 50, 70%	$z = 0.017271x + 0.017381y - 0.000149x^2$	0.68

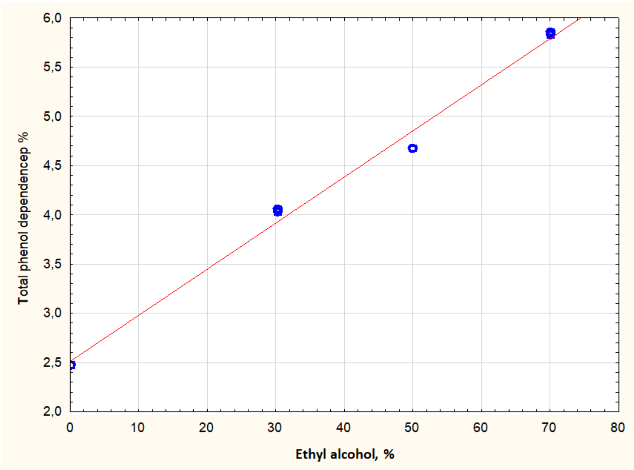

Fig. 1. Graph of the total phenol dependence in % as a function of the concentration of ethyl alcohol in %

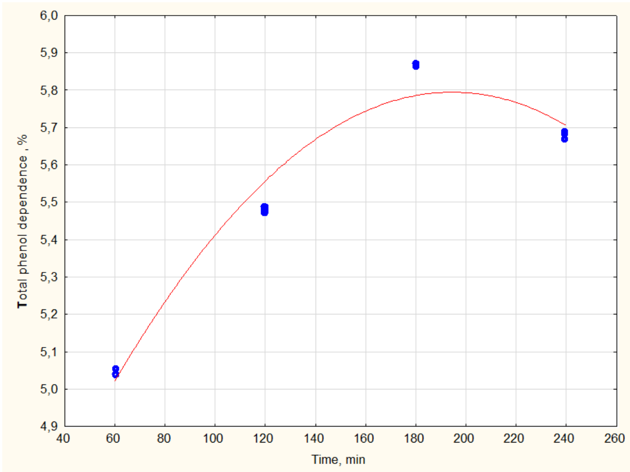

Fig. 3. Graph of total phenol dependence in %, as a function of time in min

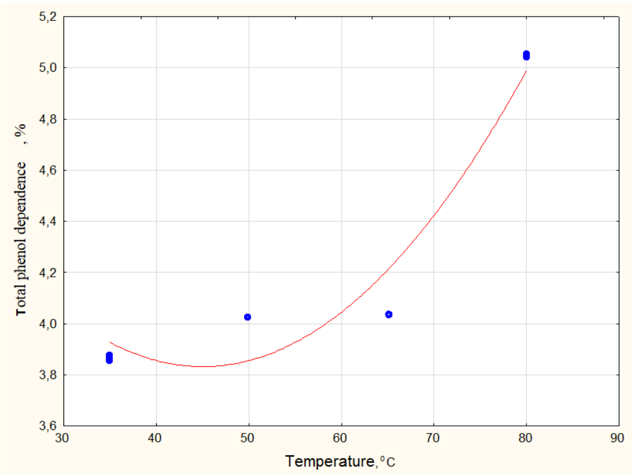

Fig. 2. Graph of the total phenol dependence in % as a function of the temperature in °C

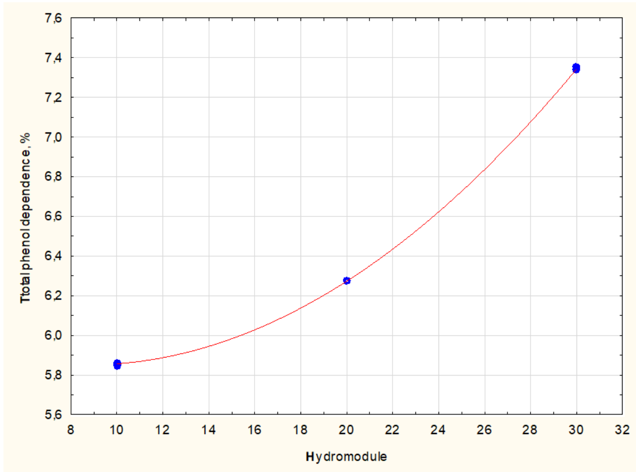

Fig. 4. Graph of the total phenol dependence in %, as a function of the hydromodule

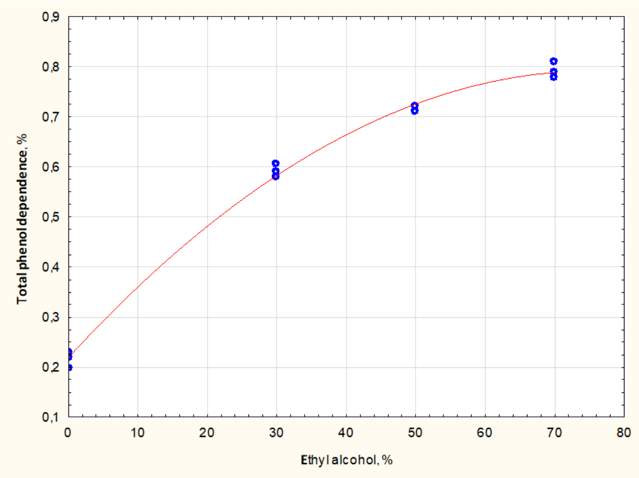

Fig. 5. Graph of the total phenol dependence in %, as a function of the concentration of ethyl alcohol in %

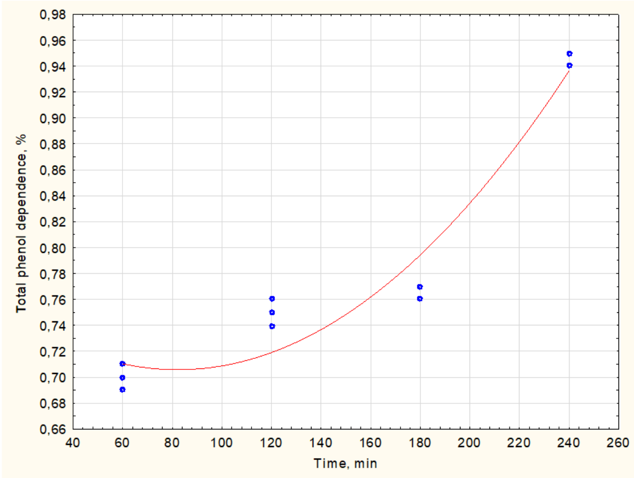

Fig. 7. Graph of the dependence of total phenols in %, as a function of time in min

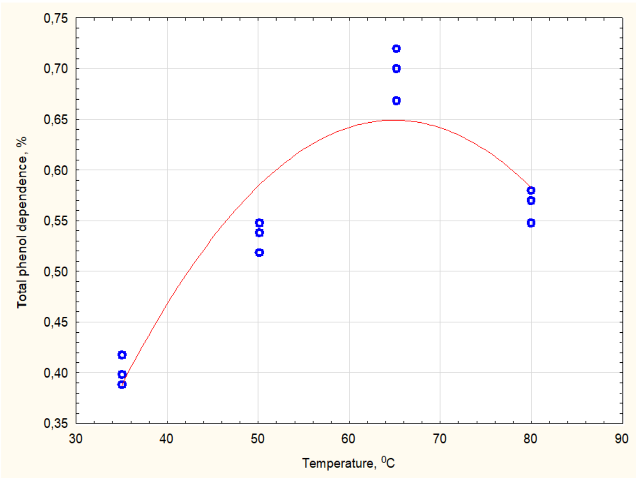

Fig. 6. Dependency graph of total phenol dependence in % as a function of temperature in 0C

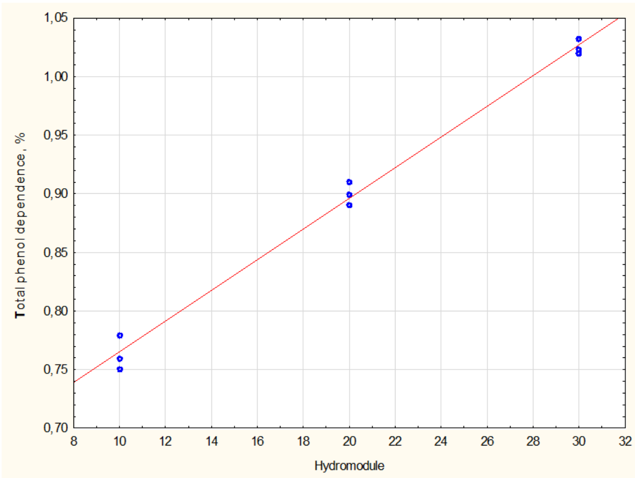

Fig. 8. Graph of the total phenol dependence in%, as a function of the hydromodule

From the graph we can conclude that the residuals are normally distributed, they are not systematic, and do not depend on the values predicted by the model (Figure 11).

From the analysis of the standardized coefficients β it is clear that the two factors, concentration and time have a relatively equal influence (Figure 12).

From the graph we can conclude that the residuals are normally distributed, they are not systematic, and do not depend on the values predicted by the model (Figure 13).

From the analysis of the standardized coefficients β, it follows that the temperature has a greater influence on the model (Figure 14).

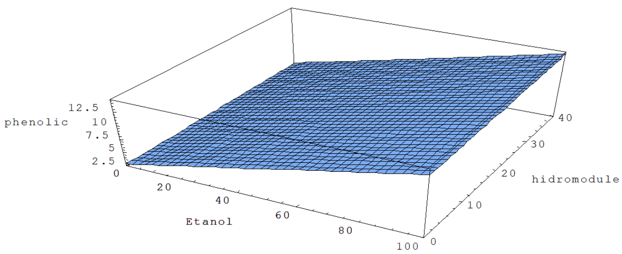

Fig. 9. Graph of the total phenol dependence in %, as a function of the hydromodule and the concentration of ethyl alcohol in %

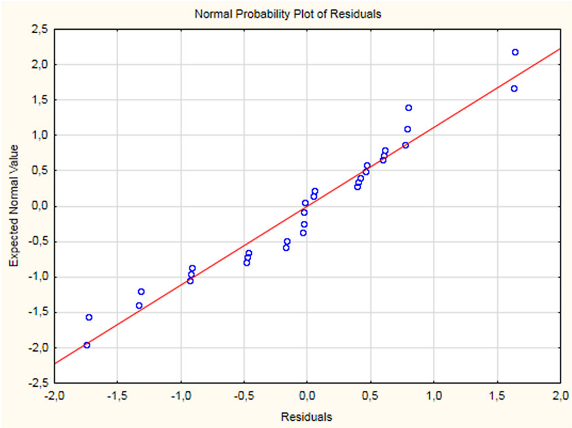

Fig. 10. Normal probability plot of residuals

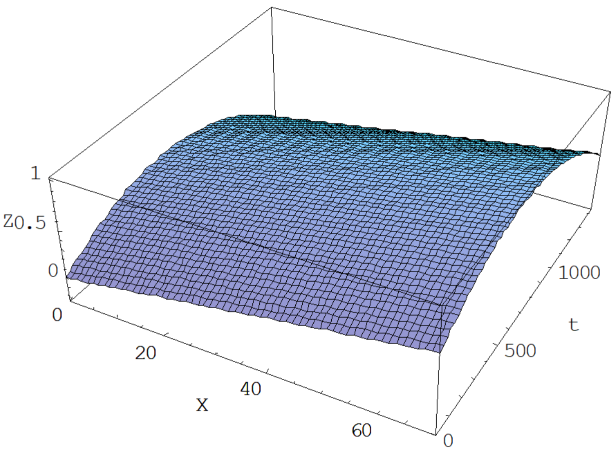

Fig. 11. Graph of total phenol dependence in %, as a function of time in minutes (t) and the concentration of ethyl alcohol in % (x)

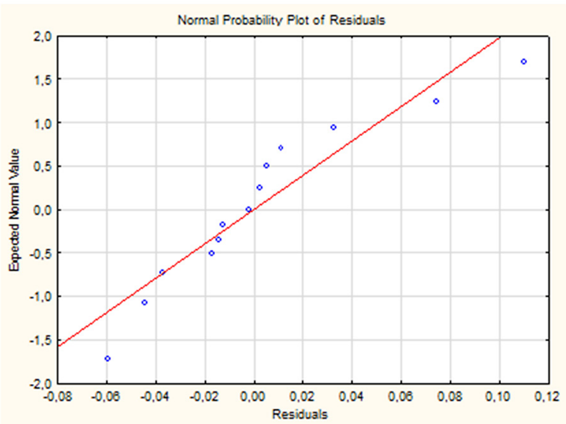

Fig. 12. Normal probability plot of residuals

From the analysis of the standardized coefficients β it is clear that the two factors, concentration and time have a relatively equal influence (Figure 15; Table 4).

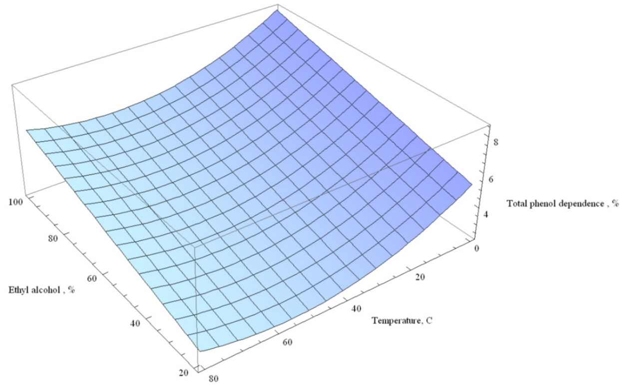

Fig. 13. Graph of total phenol dependence in % as a function of temperature in 0C and the concentration of ethyl alcohol in %

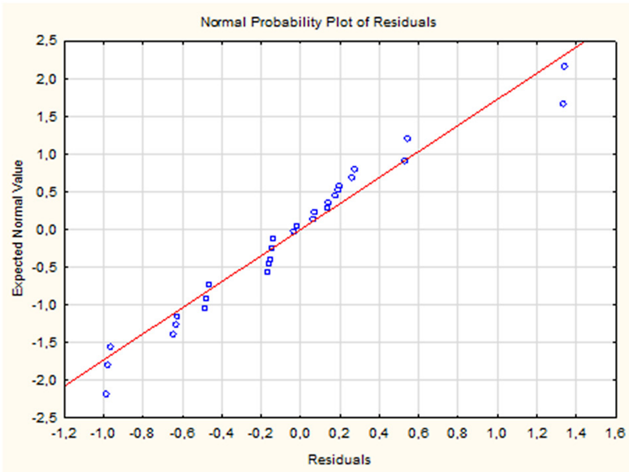

Fig. 14. Normal probability plot of residuals

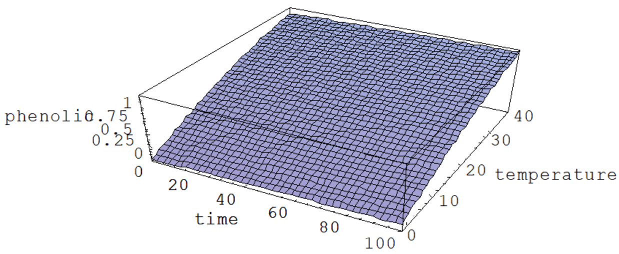

Fig. 15. Graph of total phenol dependence in% as a function of temperature in 0C and time in min

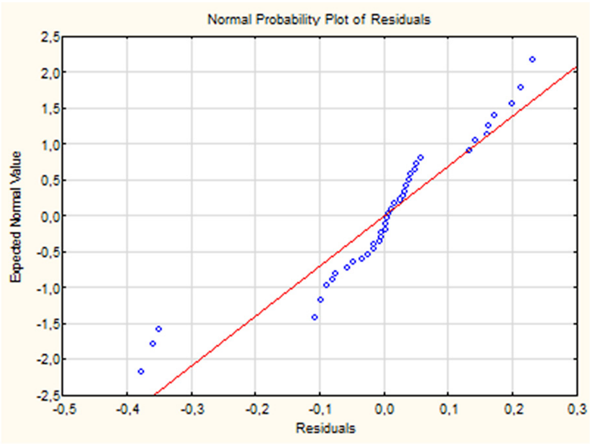

Fig. 16. Normal probability plot of residuals

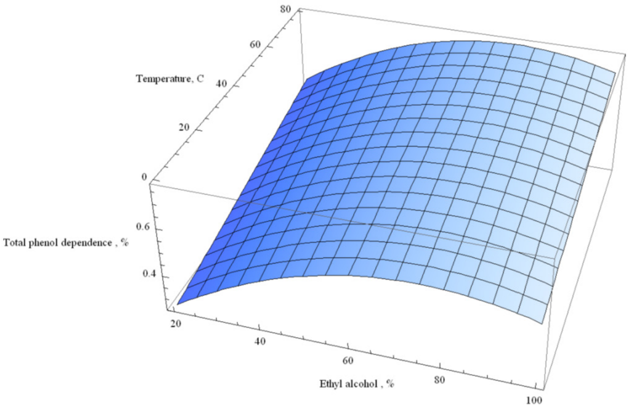

Fig. 17. Diagram of total phenol dependence in%, as a function of time in minutes and the concentration of ethyl alcohol in%

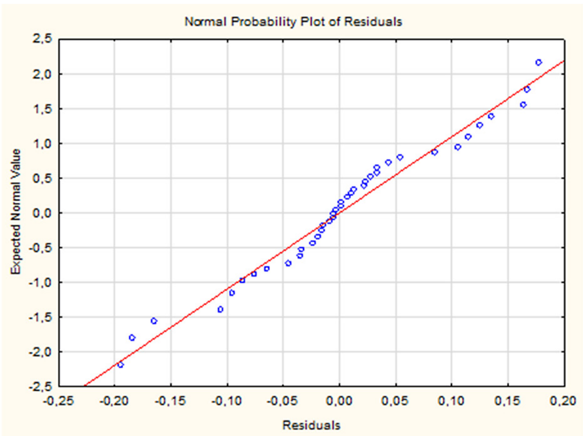

Fig. 18. Normal probability plot of residuals

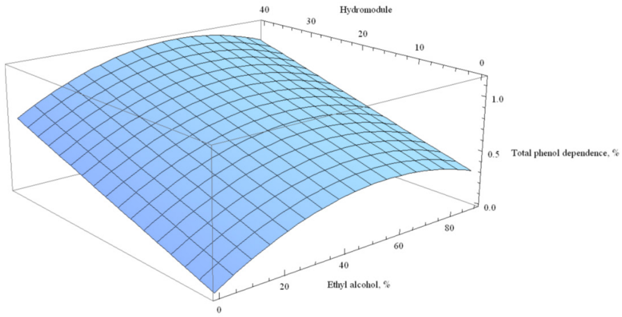

Fig. 19. Graph of the total phenol dependence in%, as a function of the hydromodule and the concentration of ethyl alcohol in%

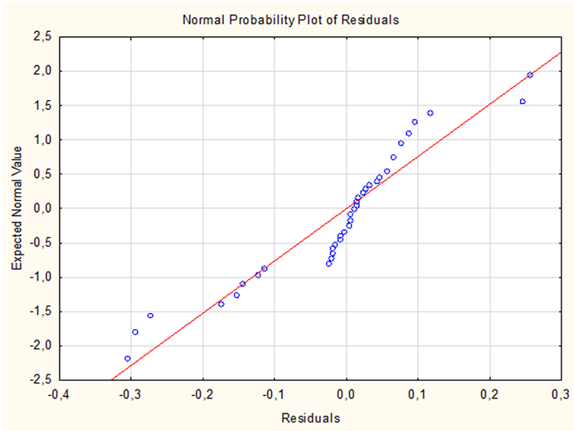

Fig. 20. Normal probability plot of residuals

From the analysis of the standardized coefficients β, it follows that the influence on the model has more time and then the temperature (Figure 16).

From the analysis of the standardized coefficients β it is clear that the two factors, concentration and time have a relatively equal influence (Figure 17).

From the analysis of standardized coefficients β, it follows that the most influence on the model have the concentration and then the temperature (Figure 18).

From the analysis of the standardized coefficients β it is clear that the two factors, concentration and time have a relatively equal influence (Figure 19).

From the analysis of standardized coefficients β, it follows that the concentration has the greatest influence on the model and then the hydromodule (Figure 20).

From the analysis of the standardized coefficients β it is clear that the two factors, concentration and time have a relatively equal influence.

Conclusions

Technology for obtaining extracts with maximum content of common phenols has been developed.

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 phenolic compounds.

Adequate mathematical models were described describing the dependencies of the individual parameters in the extraction of the common phenols.

The degree of influence of the factors on the response function has been reported.

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