

The optimization of a conventional extraction of bioactive compounds from *Cornus mas* by RSM and the determination of favourability factors by GIS technique

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Abstract

Rich in vitamins, polyphenols, carotenoids, carbohydrates, with a high antioxidant activity, cornelian cherry (*Cornus mas*) is one of the 65 species of Cornaceae family, fruits that are used in food industry, cosmetic industry or homeopathic medicine. The valuable bioactive compounds and the high economical potential of this tree makes suitable to be considered as a raw material to study the optimum extraction conditions in order to maximize the bioactive compounds' concentration. In this study, the main purpose was to investigate both the favourable and restrictive conditions for *Cornus mas* using the GIS technique. The Remote Sensing and Geographic Information Systems (GIS) technique indicated the favourability factors for *Cornus mas* trees in Romania country. The response surface methodology was employed to study the effect of temperature, time and solvent concentration on the flavonoids' (TFC) and polyphenols (TPC) concentrations and the antioxidant activity values. The maximum concentration of TPC (24.70 mg GAE/g raw material) and TFC (1.36 mg QE/g raw material) were obtained with 60% ethanol concentration at different temperature and time values. The cube model with centred faces for the Box-Behnken central composition revealed the highest value of the concentration of TAA (101.31 mg TE/g raw material) at 100% ethanol concentration, 50 °C extraction temperature and the maximum extraction time of 30 min. The most relevant variable was the solvent concentration and the obtained results suggested that the central-face cube model (86% for TPC, 97% for TFC and 95% antioxidant activity) approximated the experimental data.

Keywords: bioactive compounds; conventional extraction; *Cornus mas*; GIS technique; response surface methodology

Introduction

The current climate changes such as the migration of some species towards higher altitudes, the drying of some forest species due to longer periods of drought, the increase of the sensitivity of forest species after snow damage, windfalls, drought, or identified soil erosion both globally and regionally have a major impact on the

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vegetation. Thus, there is a need to know the areas in which certain species will reduce their coverage, but also the favourable areas where there is the possibility of their expansion appears because of climate changes with direct implications in the forest management (Schiop *et al.*, 2017; Seidl *et al.*, 2017; Todea *et al.*, 2020).

GIS technology allows the creation of a geospatial database by applying modern techniques of interpolation and spatial correlation. This includes the most relevant ecological indicators that positively and negatively influence the favourability of the species in Romania (Roşca *et al.*, 2017). As such, by analysing climatic factors, such as the multiannual average precipitations, annual average temperature, topographic characteristics (elevation), soil characteristics (soil type and geology), several thematic maps and a raster database were generated. Furthermore, by applying geospatial modelling techniques, it is possible to include these elements in a quantitative GIS model of favourability classes for *Cornus mas* (*C. mas*). According to several studies, in Romania it can be found in the spontaneous flora (Cornescu and Cosmulescu, 2017). The high amount of biologically active compounds (BACs) such as vitamins, polyphenols, carotenoids, fatty acids, anthocyanins, tannins and carbohydrates found in the *C. mas* fruits makes them valuable for the food industry, cosmetic purposes and folk medicine. Many studies present the conventional extraction performed with different solvents such as: methanol, ethanol, acetone by the methods like: maceration, ultrasound-assisted or Soxhlet extraction (Dhanani *et al.*, 2015; Altemimi *et al.*, 2017; Dumitrascu *et al.*, 2019).

C. mas fruits are used fresh or processed in food products, such as: jams, jelly, juices, liquor, marmalade, *pestil* (dried marmalade produced in Turkey), *pekmez* (syrup obtained in Turkey by fruit must), stewed fruit, syrups, tea infusions, wines (Güleryüz *et al.*, 1998; Capanoglu *et al.*, 2011; Sochor *et al.*, 2014; Nizioł-Łukaszewska *et al.*, 2018). Some fresh fruits of *C. mas* genotypes from Turkey have indicated a vitamin C content ranging from 36 to 122 mg/100 mL (Ercişli, 2004). Amongst the many benefits associated to humans health, cornelian cherry fruits are successfully used for: anaemia, cancer, diabetes, diarrhoea, fever, hyperlipidemia, gastrointestinal disorders, malaria, obesity, reducing intraocular pressure, urinary tract infections, as well as to have antioxidant and neuroprotective activity (Asgary *et al.*, 2013; Szumny *et al.*, 2015; Dinda *et al.*, 2016). The cornelian cherry forest fruits were used as the whole plant by humans over the years due to geographical distribution.

The main purpose of this study was to achieve a collection of thematic maps for the national favourability classes for *C. mas*. Further the conventional extraction was performed in order to evaluate the optimum extraction parameters in regards to three different variables (temperature, time, and hydro alcoholic solution concentration) so as to maximize the total flavonoids' content (TFC), the total polyphenols' content (TPC) and the total antioxidant activity (TAA). Our research is important due to the high occurrence of *C. mas* at a national level as raw a material and also a good perspective for it to be use as food ingredients.

Materials and Methods

GIS technique

Considering the objectives of a study, GIS technology implementation is based on a spatial analysis considering of digital databases managed through the spatial analysis functions of geoinformation programs (Roşca *et al.*, 2017).

This type of analysis techniques is used successfully in various studies to determine the most favourable agricultural crops (Islam *et al.*, 2018; Matei *et al.*, 2020), tree species (Beck *et al.*, 2020) having the advantage of providing practical landscaping solutions at different scales of analysis taking into account the landscape and soil conditions but also depending on the market requirements (Zu *et al.*, 2014). Spatial analysis allows the adaptation of crops to global climate change that imposes requirements adapted to new climatic parameters using the Analytic Hierarchy Process (AHP) system (Acharya *et al.*, 2021) the bivariate statistical analysis (BSA) or the analysis expert knowledge (Bhandari *et al.*, 2013). But this aspect involves specialized technical

knowledge, an interdisciplinary working group and the creation of a detailed database to understand the complexity of the involved ecological factors involved.

In our case, the first stage for the *C. mas* favourability determination was the acquisition of a database in a digital format (acquisition directly based on the spatial analysis sub models for the soil type and elevation, geological classes and the acquisition based on the implementation of the interpolation functions for the climatic factors).

The application of the actual spatial analysis based on the qualitative class determination through expert knowledge implies the granting of specific classes of favourability as well as the restrictiveness for the species studied within the entire territory of Romania (Table 1). It also implies as the final step - absolutely compulsory for the GIS models - the model validation stage (by directly comparing it to the reality from the field) in order to determine the predictability degree of predictability of the created model and the possible calibration of its parameters (Roşca *et al.*, 2019).

Table 1. Favourability classes of the ecological factors used for the modelling of *C. mas*

Factor	Favorability for <i>C. mas</i>			Sources
	Low	Medium	High	
Elevation (m)	<100	>800	100.1-800	Copernicus Land Monitoring Service - EU-DEM (EEA)
Air temperature (°C)	<-2	-2...10.5	>10.5	Implementation in GIS environment of the regression equation: Y = 0.395 X + 363.76 where: Y - the average of the annual precipitation, mm X - the average of the annual temperature, °C
Average precipitations (mm/year)	900 mm/year	650-900 mm/year	527-650 mm/year	Implementation in GIS environment of the regression equation: Y = 0.435 X + 533.51, where: Y - the average of the annual precipitation, mm X - the relative altitude, m
Type of soil	Cambic Chernozem, erodosoils, alluvial soils	Cernozioms clayoiluvial, lacovisti, podzoles, regosols, vertisols	Litosols, Rendzine, Terra-rossa, Cernozioms, Preluvosols, Faeoziom, Cernozioms	Soils Map of Romania, 1: 200000
Geology	Breccias, salt diapir	Gravels, flis deposits, conglomerates, coluvial deposits, alluvial deposits, flish deposits	Andesite, clays, leosoid deposits, limestone, marls, sandy deposits, tephogen rocks, current alluvials, limestone	Geological Map of Romania, 1:200000

The validation of the results was performed through the comparison of the results obtained by applying the model developed in this study to frequency points reported in the European Atlas of Forest Species in Europe in the year of 2016.

Chemicals and reagents

Ethanol, aluminium chloride, potassium acetate, Folin-Ciocalteu reagent, sodium carbonate, gallic acid, methanol, (1,2-Diphenyl-1-picrylhydrazyl) DPPH, (6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic

acid) Trolox were purchased Sigma-Aldrich (Steinheim, Germany). The chemicals used in this work were of analytical or HPLC grade.

Sample processing

The cornelian cherry fruits were collected from spontaneous flora from different village's hills of the Galati County, Romania, in September 2017. After being washed with distilled water, the fruits were blotted on paper towels and the stone of the fruits were removed. Afterwards, the fruits were freeze-dried (Christ Alpha 1-4 LD plus, Germany) at -42 °C under a pressure of 0.10 mbar for 72 h. In order to obtain a compact powder, after lyophilization, the fruits were grinded under domestic conditions and stored at 4 °C for further experiments. In addition, 100 fruits and stones of cornelian cherry were measured and weighed in order to make a correlation between them and the characteristics of the fruit collection area used in this study.

Conventional extraction method and the phytochemical characterization of the extracts

The Design Expert software was used to analyse the influence of three different variables regarding the conventional extraction (CE) method (temperature 30-50 °C, time 15-45 min and ethanol concentration 60-100%). In order to maximize the BACs content, the BOX-Behnken model with 15 experiments was used and for each experiment 1 g of lyophilized raw material and 10 ml solvent were used. The total flavonoids content (TFC) was determined using a modified colorimetric method described by Turturică *et al.* (2016). The total polyphenols content (TPC) was determined using Folin-Ciocalteu method, expressed as mg Gallic Acid Equivalent (GAE)/g raw material while the total antioxidant activity (TAA), expressed as mg Trolox/g raw material described as described by Vasile *et al.* (2020) and Oancea *et al.* (2017).

All the experiments were done using a water bath (Grant, OLS 200). All the measurements were replicated three times and the responses experimental values were expressed as the means.

Response surface methodology

The Response Surface Methodology (RSM) was applied to obtain the optimum conditions for the maximum extraction values of the TPC, the TFC and the TAA. The Box-Behnken design (BBD) was used to determine the optimum conditions for the CE method.

For the CE the three independent variables, namely extraction temperature (°C, X_1), extraction time (min., X_2) and solvent concentration (%EtOH, X_3) at three levels (-1,0,+1) were investigated (Nipornram *et al.*, 2018).

The coded and decoded values of the used independent variables used and their levels are shown in Table 1 and Table 2. The designs consisted of 15 randomized runs with three replicates at the central point and were analysed using the Design-Expert 6.0 Trial software (Stat-Ease, Minneapolis, Minnesota, USA) in terms of statistical analysis of variance, regression coefficients and regression equation. The experimental data for the three responses were fitted into a second-order polynomial model based on Eq. (1) in terms of the code factors, as follows:

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=2}^3 \beta_{ij} X_i X_j \quad (1)$$

where Y represents the response variable, X_i and X_j are the independent variables affecting the responses, and β_0 , β_i , β_{ii} and β_{ij} are the regression coefficients for the intercept, linear, quadratic and interaction terms (Zeković *et al.*, 2014).

The optimum extraction conditions were determined considering the highest values for the total flavonoids, total polyphenols and the antioxidant activity values.

Statistical analysis

The Design-Expert 6.0 Trial (Stat-Ease, Minneapolis, Minnesota, USA) software was used for the Response Surface Methodology. The statistical analysis of the data was performed using ANOVA.

Results and Discussion

GIS technique

To the best of our knowledge, in Romania only 38 areas were identified where the *C. mas* trees can grown, as described in Table 2 (Doniță *et al.*, 2005).

Table 2. Romania's areas of cornelian cherry trees (Doniță *et al.*, 2005)

R3115	R3129	R4114	R4126	R4133	R4137	R4147	R4153	R4159	R4164
R3122	R3130	R4120	R4127	R4134	R4140	R4149	R4154	R4160	R4218
R3126	R4111	R4121	R4129	R4135	R4141	R4150	R4156	R4162	
R3128	R4113	R4124	R4132	R4136	R4142	R4151	R4158	R4163	

- 1.R3115 South-East Carpathian bushes of *Juniperus sabina*
- 2.R3122 Ponto-Pannonic bushes of *Prunus spinosa* and *Crataegus monogyna*
- 3.R3126 South-East Carpathian bushes of *Fraxinus ornus*
- 4.R3128 Balkan bushes of *Paliurus spina-christi*
- 5.R3129 Balkan bushes of *Jasminum fruticans*
- 6.R3130 Ponto-Pannonic bushes of *Cerasus fruticosa*
- 7.R4111 South-East Carpathian forests of *Fagus sylvatica* and *Abies alba* with *Cephalanthera damasonium*
- 8.R4113 Balkan forests of *Fagus sylvatica* with *Helleborus odoratus*
- 9.R4114 Mixed Balkan forests of *Fagus sylvatica* with *Ruscus saculatus*
- 10.R4120 Mixed Moldavian forests of *Fagus sylvatica* and *Tilia tomentosa* with *Carex brevicollis*
- 11.R4121 Balkan forests of *Fagus sylvatica* and *Corylus colurna* with *Knautia drymeia*
- 12.R4124 Dacian forests of *Quercus petraea*, *Fagus sylvatica* and *Carpinus betulus* with *Lathyrus hallersteinii*
- 13.R4126 Mixed Moldovan forests of *Quercus petraea*, *Fagus sylvatica* and *Tilia tomentosa* with *Carex brevicollis*
- 14.R4127 Mixed Dacian forests of *Quercus petraea*, *Fagus sylvatica* and *Tilia tomentosa* with *Erythronium dens-canis*
- 15.R4129 Dacian forests of *Quercus petraea*, *Fagus sylvatica* with *Festuca drymeia*
- 16.R4132 Pannonic-Balkan forests of *Quercus petraea* and *Quercus cerris*, *Fagus sylvatica* with *Melittis melissophyllum*
- 17.R4133 Balkan forests of *Quercus petraea* with *Helleborus odoratus*
- 18.R4134 Western-Pontic forests of *Quercus petraea* with *Mercurialis ovata*
- 19.R4135 West-Pontic mixed forest of *Quercus petraea*, *Tilia tomentosa* and *Carpinus betulus* with *Carpesium cernuum*
- 20.R4136 Mixed West-Pontic forests of *Quercus petraea*, *Tilia tomentosa* and *Carpinus orientalis* with *Nectaroscordum siculum*
- 21.R4137 Mixed West-Pontic forests of *Quercus petraea* and *Tilia tomentosa* with *Galanthus plicatus*
- 22.R4140 Daco-Balkan forests of *Quercus petraea*, *Quercus cerris* and *Tilia tomentosa* with *Lychnis coronaria*
- 23.R4141 Daco-Balkan forests of *Quercus petraea* and *Castanea sativa* with *Genista tinctoria*
- 24.R4142 Mixed Balkan forests of *Quercus petraea* and *Corylus colurna* with *Paeonia dahurica*
- 25.R4147 Mixed Danubian forests of *Quercus robur* and *Tilia tomentosa* with *Scutellaria altissima*
- 26.R4149 Danubian-Balkan forests of *Quercus cerris* with *Pulmonaria mollis*
- 27.R4150 Danubian-Balkan forests of *Quercus cerris* with *Festuca heterophylla*
- 28.R4151 Mixed Balkan forests of *Quercus cerris* with *Lithospermum purpureocaeruleum*
- 29.R4153 Danubian-Balkan forests of *Quercus cerris* and *Quercus frainetto* with *Crocus flavus*

- 30.R4154 Danubian-Balkan forests of *Quercus frainetto* with *Festuca heterophylla*
- 31.R4156 Danubian-Balkan forests of *Quercus pedunculiflora*, *Quercus cerris*, *Quercus frainetto*, *Quercus pubescens* with *Acer tataricum*
- 32.R4158 Mixed Danubian-West-Pontice forests of *Quercus pedunculiflora* and *Tilia tomentosa* with *Viola jordanii*
- 33.R4159 Danubian forests and rarities of *Quercus pedunculiflora* and *Quercus robur* with *Tulipa bibersteiniana*
- 34.R4160 Dacian forests-rarities of *Quercus pubescens* with *Lithospermum purpurocaeruleum*
- 35.R4162 Mixed West-Pontic forests of *Quercus pubescens* with *Paeonia peregrina*
- 36.R4163 Balkan forests-rarists of *Quercus pubescens* with *Echinops bannaticus*
- 37.R4164 Balkan forests of *Juglans regia* and *Celtis australis* with *Scutellaria picheleri*
- 38.R4218 Forests-rarists South-East carpathic of *Pinus nigra* spp. *banatica* with *Genis taradiat*

Favourability of the environmental factors for C. mas trees in Romania

Taking into account the preferences of the *C. mas* species, a database was created with the multiannual precipitations values from the meteorological stations in Romania. Based on the GIS spatial analysis technique and by using the equations between the precipitations level and the elevation, the grid of the multiannual average precipitations was obtained. This was converted by considering the favourability as well as the restrictiveness of the studied species.

Thus, the territories that benefit from values of an average multiannual precipitations lower than 650 mm/year were included in the classes of high favourability since the species has low requirements for water. The areas characterized by precipitation values between 650-900 mm/year were included in the medium favourability class. Thus, the main part of the Transylvanian Plateau, the area of the Sub-Carpathian hills, but also that of the low mountains offer medium conditions to this species from the pluviometry point of view.

In the areas where the volume of precipitations surpasses 900 mm/year, the restrictive conditions were fulfilled for *C. mas*, therefore the high-mountainous area of the Romanian Carpathians was included in the low favourability classes (Figure 1).

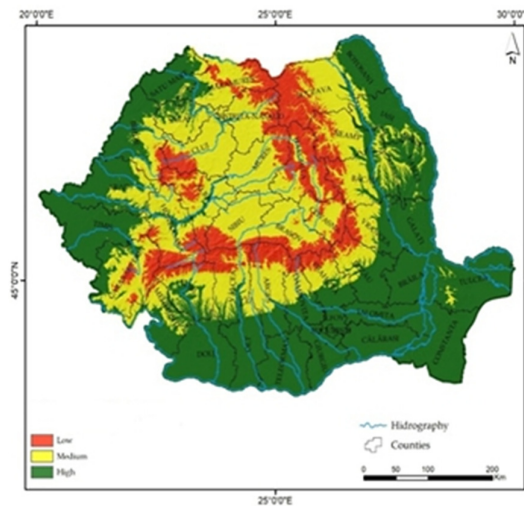


Figure 1. Favourability map for *Cornus mas* depending on the annual average precipitations

In Europe, according to the field observations taken by experts, the highest concentration of the species was identified in territories characterised by precipitation values between 500-800 mm/year and in the territories with air temperatures in the range of 8-12 °C and with the solar radiation values during the spring-summer season between 1500-1700 (kWh m²) (San-Miguel-Ayanz *et al.*, 2016).

By using the same procedural stages, the multiannual average temperature grid was obtained which was the basis in generating the map of favourability map for *C. mas* in terms of the air temperature (Figure 2). Thus, it could be observed that that the largest part of the Romanian territory offers medium favourable conditions for *C. mas*. Only the areas of the Danube floodplain, the Bărăgan Plain and the seaside are characterised by temperatures higher than 10.5 °C. As such, these are characterised by the best development conditions of the species, whereas the limitative areas were identified in the high-mountainous areas, characterised by temperature values that can reach 2 °C.

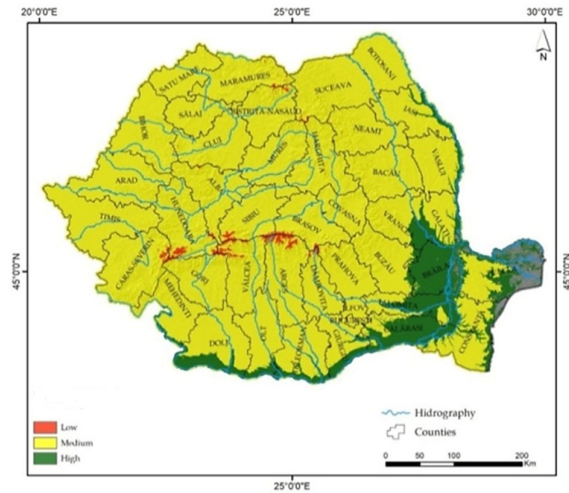


Figure 2. Favourability for *Cornus mas* depending on the air temperature

Considering the influence of the elevation on the precipitations' volume, on the air temperature and, implicitly, on the pedological characteristics, and by using the information provided by the scientific literature on the ecological requirements of the analysed species, the Romanian territory was classified based on three favourability classes. The areas with an elevation lower than 100 m offer limitative conditions for the cultivation of *C. mas*, whereas the areas within the elevation 100-800 m offer good development conditions. Thus, the high areas characterised by a higher elevation than 800 m may become restrictive to the development and the achievement of an increased consistency of the species.

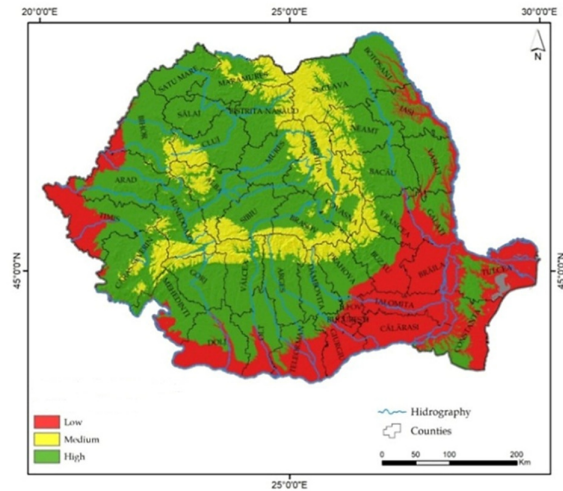


Figure 3. Favourability for *C. mas* depending on the elevation

The analysis of the forest habitats' types from Romania, where *C. mas* grows, allowed a classification based on favourability classes correlated to the soil types (Figure 3, Table 1). It can be notice that a considerable part of the Romanian territory (143010 km² representing 59.85%) offers favourable conditions for the development of the species through the presence of specific soil types: Lithosols, Rendzinas, Terra-rossa, Chernozem, Preluvosoil, Phaeozem. The restrictive conditions are fulfilled on an approximately 25.16% of the national territory where types of soil such as Cambic Chernozem, Erodosoils, alluvial soils are present, whereas the other 14.9% of the territory offers medium conditions for the analysed species (Figure 4).

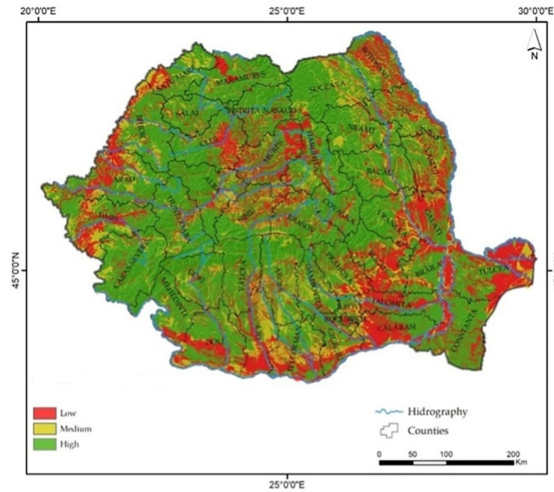


Figure 4. Favourability for *Cornus mas* depending on the type of soil

Another factor taken into consideration was the geological deposit. Each geological class mapped on the Geological Map of Romania 1:200000 was classified by the favourability classes for *C. mas* considering the geology of forest habitats at the level of which this species was identified (Table 1). Therefore, a large part of the Romanian territory (58.9% of its surface) offers favourable conditions for *C. mas*. Only 4.2% imposes limits within the territories with salt deposits whereas the other territories offer medium development conditions for the studied species (Figure 5).

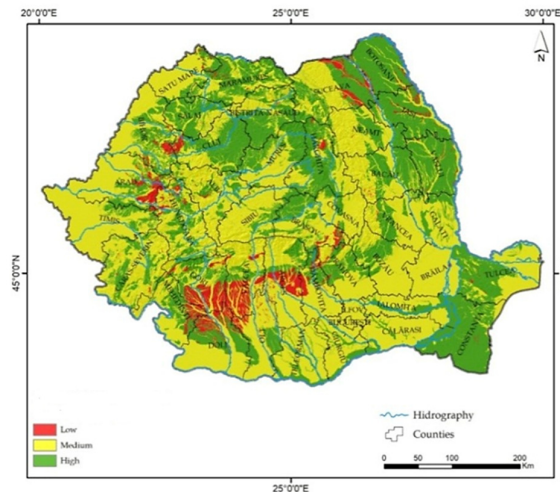


Figure 5. Favourability for *C. mas* depending on geology

At a national level, the GIS analysis has highlighted the medium and the high favourability classes for *C. mas* which represented 75% of the studied territory, due to climatic, soil and geological conditions (Figure 6).

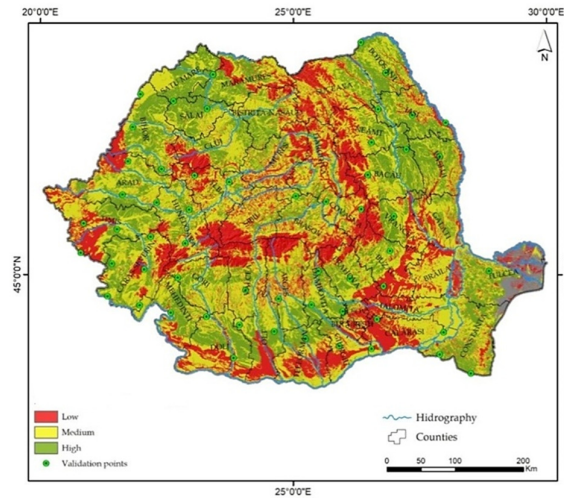


Figure 6. Favourability for *C. mas* depending on all the parameters (climatic, soil and geological conditions)

The largest areas that can support the development of the species and the obtainment of abundant fructification are located in several counties, such as: Bihor, Constanța and Bacău, whereas the most restrictive areas are located in the following counties: Suceava, Timiș, Harghita etc. (Table 3).

The validation of the model was made by using the reclassified result obtained from the implementation of a spatial analysis (favourability or restrictiveness for *C. mas* and a vector database of a polygon type. This was obtained from a direct acquisition representing geographical locations of the areas where the species exceeds 25% of the consistency of the habitats in which it develops, according to the Atlas of Species in Europe. Therefore, a validation rate of 83.5 % of the results was identified, which reflects the high level of sensitivity of the applied model (28 safe locations in the field where the *C. mas* species was identified, located in areas are included in the high favourability class, whereas 19 are present in the medium favourability class).

Response Surface Methodology for the CE method of the BACs from cornelian cherry fruits

The experimental values were fitted to a second-order polynomial model (Eq.1) and the multiple regression coefficients were generated for all the responses using a statistical approach to determine the influence of the extraction parameters on the BACs from *C. mas* fruits.

The highest values for the BACs were obtained at 40 °C, 15 min and 60% hydro alcoholic solution, a Gallic Acid Equivalent GAE, 24.70±0.92 mg/g raw material, a Quercetin Equivalent QE, 1.36±0.00 mg/g raw material was obtained at 30 °C, 30 min and 60% hydro alcoholic solution, whereas the total antioxidant activity, of 101.31±3.90 mg Trolox /g raw material, was detected at 50 °C, 30 min and 100% etanol (EtOH).

Table 3. Classification based on the favourability classes for *C. mas* in the Romanian counties

County	Favourability Classes Area (km ²)		
	Low	Medium	High
Alba	2120.4	3531.5	597.2
Arad	1226.6	4122.5	2399.8
Arges	2103.3	3542.6	1184.8
Bacău	1165.9	2715.0	2746.7
Bihor	1007.9	3338.4	3202.5
Bistrita Năsăud	838.6	2795.8	1724.7
Botosani	380.3	2178.1	2385.2
Brăila	1527.7	3103.8	0.9
Brasov	1748.3	2647.3	972.4
Bucuresti	137.8	95.7	2.3
Buzău	2530.1	2634.0	943.9
Călărași	2283.1	2675.5	131.6
Caras_Severin	1594.4	4230.9	2697.9
Cluj	1344.5	3501.6	1833.0
Constanta	674.4	3093.9	3026.6
Covasna	1515.7	1898.5	297.6
Dâmbovita	388.1	1880.4	1789.3
Dolj	1927.4	3225.0	2261.5
Galati	1029.8	2328.6	1052.5
Giurgiu	1180.2	1864.9	499.9
Gorj	903.0	3091.2	1511.0
Harghita	2719.1	3278.0	646.6
Hunedoara	1849.0	3808.5	1412.9
Ialomita	1392.8	2811.5	256.0
Iasi	849.7	2178.5	2435.1
Ilfov	776.7	629.0	166.0
Maramures	1243.1	2482.6	2580.7
Mehedinti	678.8	2374.7	1894.4
Mures	2467.5	3285.7	965.6
Neamt	1585.0	2751.1	1569.8
Olt	1573.3	2189.1	1744.8
Prahova	1440.1	1970.8	1309.2
Sălaj	61.2	1617.2	2190.4
Satu_Mare	50.2	1850.8	2504.3
Sibiu	2254.3	2747.4	434.6
Suceava	3099.0	4079.6	1377.1
Teleorman	1958.1	2666.5	1165.5
Timis	2744.4	3815.8	2138.2
Tulcea	1128.8	2332.3	2080.1
Vâlcea	1941.4	3087.8	827.0
Vaslui	591.4	2085.6	2647.5
Vrancea	1601.9	1883.3	1375.9
Total area	59633.2	112421.2	66335.8
	25,0 %	47,2%	27,8%

Table 4. The experimental data for the TPC, TFC and TAA by CE method using the Box-Behnken model

Run	Extraction conditions						Response variables, as mean \pm S.E. (in mg/g raw material)		
	Coded variables			Decoded variables			GAE	QE	Trolox
	X ₁	X ₂	X ₃	Temperature, °C	Time, min.	EtOH, %			
1	-1	-1	0	30	15	80	17.65 \pm 0.17	1.04 \pm 0.07	10.03 \pm 1.90
2	1	-1	0	50	15	80	16.08 \pm 0.25	1.09 \pm 0.05	23.53 \pm 3.31
3	-1	1	0	30	45	80	14.66 \pm 0.64	0.93 \pm 0.08	21.19 \pm 0.02
4	1	1	0	50	45	80	10.61 \pm 0.49	1.10 \pm 0.05	56.03 \pm 3.70
5	-1	0	-1	30	30	60	23.31 \pm 0.31	1.36 \pm 0.00	13.62 \pm 3.39
6	1	0	-1	50	30	60	22.51 \pm 0.44	1.18 \pm 0.01	8.38 \pm 0.88
7	-1	0	1	30	30	100	0.89 \pm 0.06	0.23 \pm 0.02	98.48 \pm 1.71
8	1	0	1	50	30	100	1.52 \pm 0.16	0.08 \pm 0.00	101.31 \pm 3.90
9	0	-1	-1	40	15	60	24.70 \pm 0.92	1.30 \pm 0.01	9.17 \pm 2.86
10	0	1	-1	40	45	60	19.13 \pm 1.13	1.17 \pm 0.00	10.97 \pm 1.72
11	0	-1	1	40	15	100	2.12 \pm 0.08	0.19 \pm 0.01	91.93 \pm 1.27
12	0	1	1	40	45	100	1.66 \pm 0.08	0.32 \pm 0.03	88.77 \pm 1.15
13	0	0	0	40	30	80	8.21 \pm 0.13	0.89 \pm 0.00	48.35 \pm 4.83
14	0	0	0	40	30	80	8.03 \pm 0.08	0.97 \pm 0.07	56.18 \pm 0.92
15	0	0	0	40	30	80	13.23 \pm 0.17	1.07 \pm 0.06	37.22 \pm 4.31

The regression coefficients of the fitted second-order polynomial model for the BACs expressed as GAE, QE and Trolox Equivalents are presented in Table 5.

Table 5. The estimated coefficients of the fitted second-order polynomial model for the analysed BACs

Regression Coefficients	Response, mg/g raw material		
	GAE	QE	Trolox
β_0	14.278	-0.029	-8.887
A. Temperature, °C	-2.123	-0.021	4.4303
B. Time, min.	-0.933	0.037	2.5691
C. EtOH concentration, %	-0.579	0.077	-4.3093
AB	-0.004	0.0002	0.0355
AC	0.002	0.00004	0.010
BC	0.004	0.0002	-0.0041
A ²	0.025	0.00013	-0.0716
B ²	0.010	0.00020	-0.0551
C ²	0.0007	0.00069	0.0384

The analysis of variances (ANOVA) of the fitted second-order polynomial model for the TPC, TFC and TAA responses is summarized in Table 6.

The high values of coefficients of multiple determination for all BACs ($R^2_{\text{TPC}} = 0.86$, $R^2_{\text{TFC}} = 0.97$ and $R^2_{\text{TAA}} = 0.95$, respectively) through the model equations provided a good representation of the experimental values. For all three responses (the TPC, the TFC and the TAA), the mathematical models were statistically acceptable due to a significant regression for the model, $p < 0.05$ (Table 6). For this model, the lack-of-fit values confirmed the adequacy of the experimental data fitting with insignificant p -values were ($p > 0.05$).

Table 6. Analysis of variances (ANOVA) of the fitted second-order polynomial model for TPC, TFC and TAA

	Sum of squares	DF	Mean square	F-Value	p-Value
TPC (GAE ^a , mg/g)					
Model	952.88	9	105.88	22.71	0.0015 ^s
Residual	23.31	5	4.66		
Lack of fit	5.87	3	1.96	0.2243	0.8737 ^{ns}
Pure error	17.44	2	8.72		
Total	976.19	14			
TFC (QE ^b , mg/g)					
Model	2.52	9	0.2795	21.26	0.0018 ^s
Residual	0.0657	5	0.0131		
Lack of fit	0.0490	3	0.0163	1.95	0.3568 ^{ns}
Pure error	0.0168	2	0.0084		
Total	2.58	14			
Total Antioxidant Activity TAA (mg Trolox/g ^c)					
Model	16692.25	9	1854.69	11.67	0.0073 ^s
Residual	794.48	5	158.90		
Lack of fit	613.01	3	204.34	2.25	0.3222 ^{ns}
Pure error	181.47	2	90.73		
Total	17486.72	14			

^aThe coefficient of determination (R^2) of the model was 0.86;

^bThe coefficient of determination (R^2) of the model was 0.97;

^cThe coefficient of determination (R^2) of the model was 0.95;

^s – significant;

^{ns} – not significant.

The GAE response was not significantly influenced by the combined action of the time-temperature parameters. A decrease in the TPC was observed at 40 °C and 30 minutes. The use of the temperature of 30 °C and an extraction time in the range between 15 and 45 min allowed a satisfactory concentration in the TPC (Figure 7A). The influence of the combined action of the EtOH- extraction temperature parameters revealed a considerable increase of the TPC at the concentration of 60% EtOH throughout the temperature range between 30 to 50 °C (Figure 7B). Using an optimum of EOH concentration of 60%, the optimum of extraction time for the temperature range between 30-50 °C, was maximum 15 minutes. Any increase in time over 15 minutes led to a decrease of the TPC (Figure 7C).

The QE response was not significantly influenced by the combined action of the time-temperature parameters. A decrease of the TFC was observed at 40 °C and 30 minutes of extraction. The use of the temperature of 30 °C and an extraction time between 15-30 min will allow a satisfactory concentration in QE (Figure 8A). The influence of the combined action of the EtOH - temperature parameters revealed a considerable the TFC increase at an EtOH concentration in the range 60-80% throughout the temperature range between 30-50 °C (Figure 8B). Using an EtOH concentration between 60-70% in the temperature range between 30-50 °C, the optimum extraction time was 15 minutes. Any increase in time over 15 minutes leads to a decrease in the concentration of the TFC content (Figure 8C).

The TAA response was influenced by the combined action of the time-temperature parameters. The use of a temperature found in the 40 °C to 50 °C range and a time interval of between 30-45 min., will allow a satisfactory TAA to be obtained. The use of a higher temperature revealed a better resistance of the phenolic compounds expressed as Trolox Equivalents (Figure 9A). The influence of the combined action of the EtOH-temperature concentration parameters revealed a considerable increase of the TAA at a 95-100% co-solvent EtOH concentration throughout the temperature range between 30 °C to 50 °C (Figure 9B).

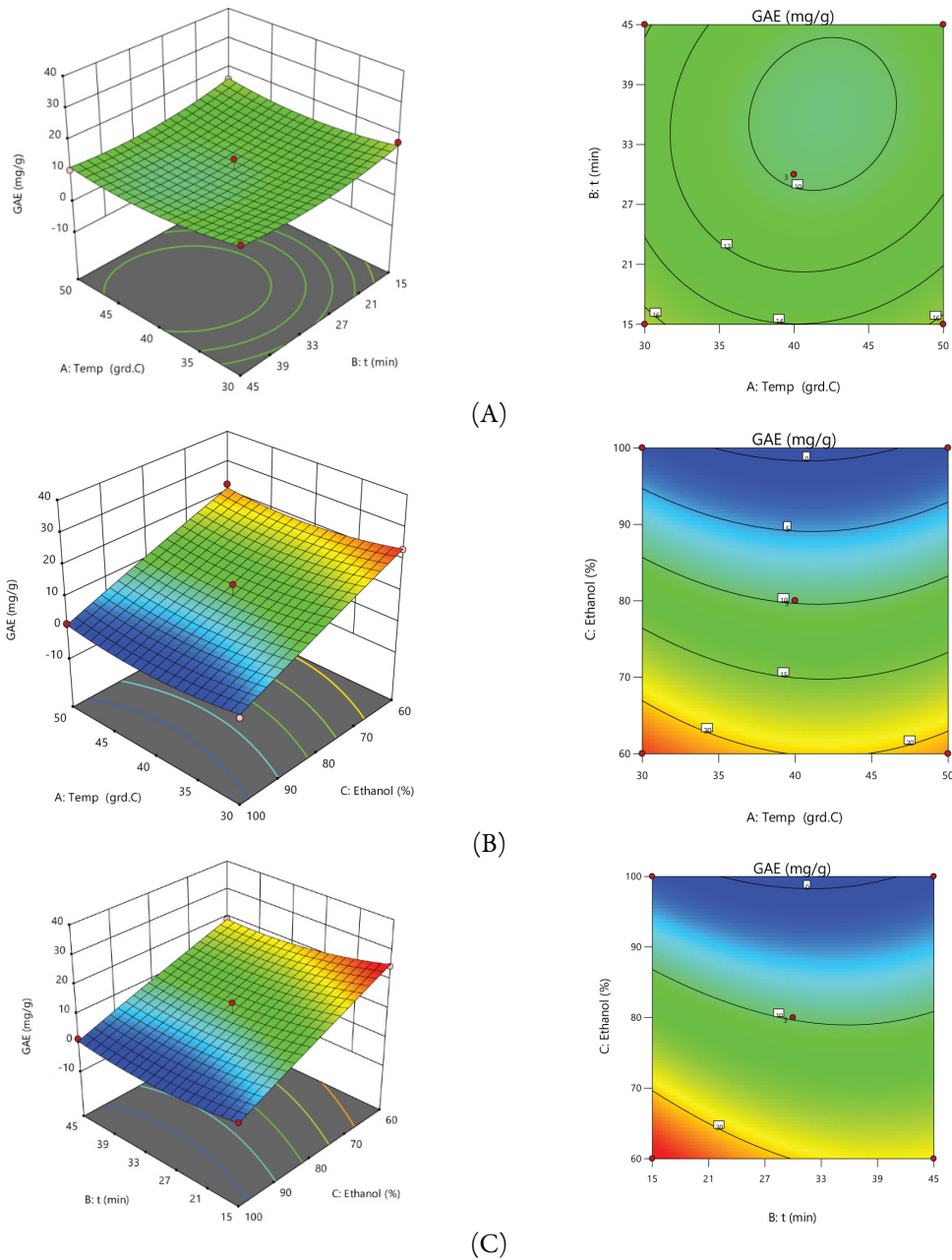


Figure 7. Response surface and contour plot that share the combined effect of temperature (A), time extraction (B) and EtOH concentration (C) on the TPC content

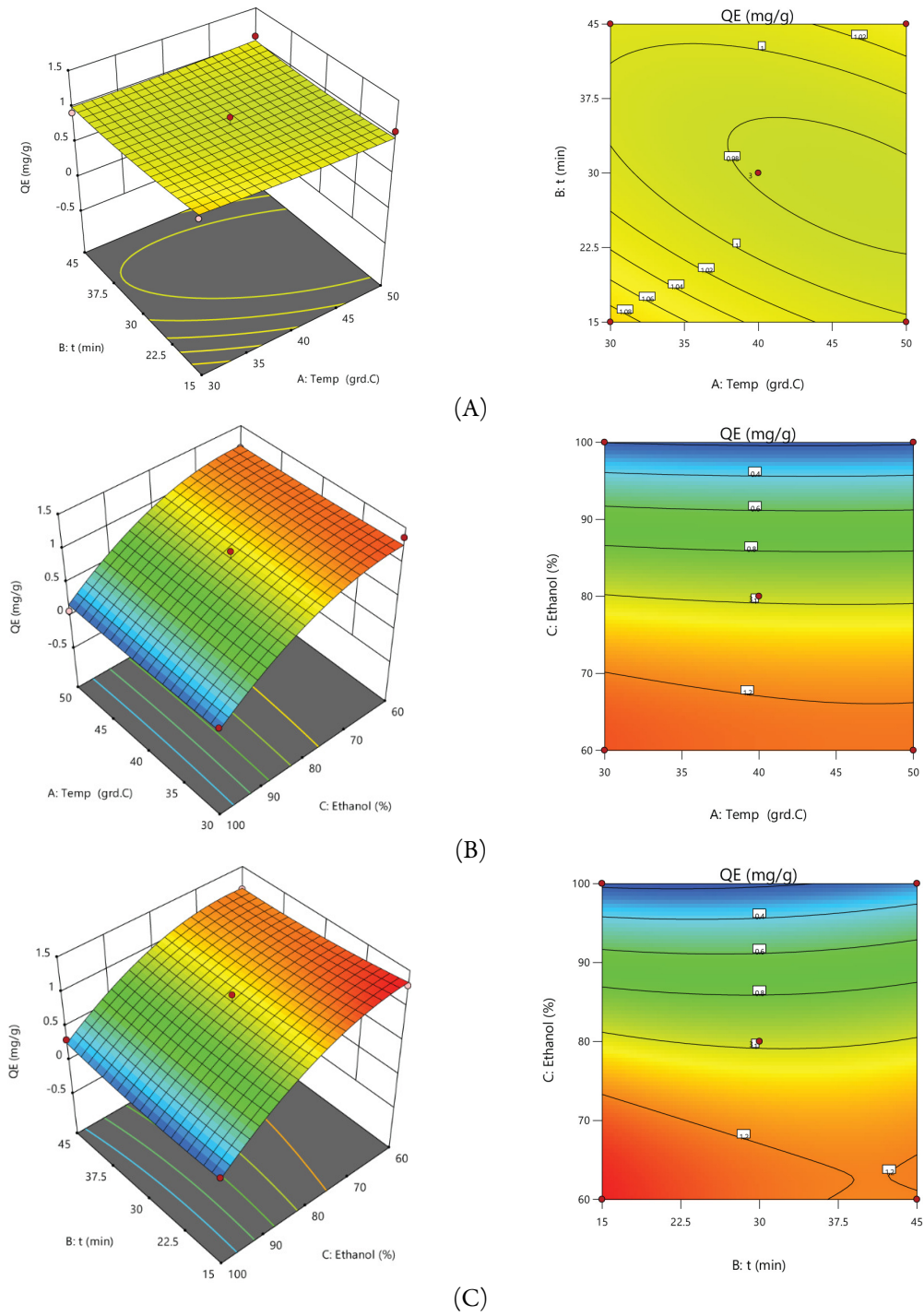


Figure 8. Response surface and contour plot that share the combined effect of temperature (A), time extraction (B) and EtOH concentration (C) on the TFC content

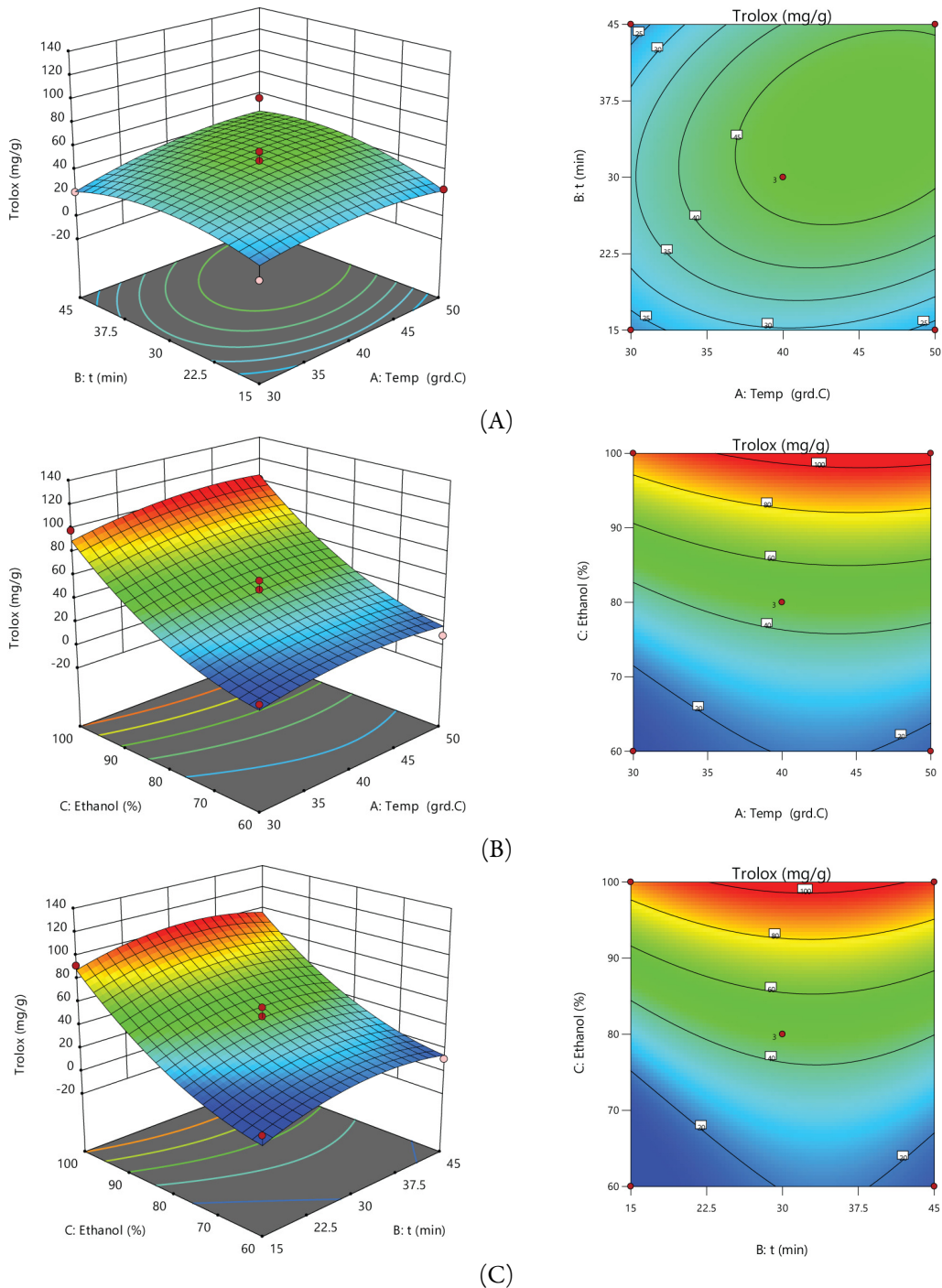


Figure 9. Response surface and contour plot that share the combined effect of temperature (A), time extraction (B.) and EtOH concentration (C) on TAA

Using the optimum concentration of 100% EtOH, the optimum extraction time for the temperature range of 30-50 °C is 15-45 minutes. A maximum concentration was reached after 30 minutes of extraction (Figure 9C).

The experimental values (observed values) and those predicted for the TPC are found in the immediate vicinity of the correlation line, which shows that the model predicted the experimental data very well, fact also

proven by the coefficient of determination value $R^2=0.86$ (quadratic model). This shows that 86% of the experimental data are approximated by the model. The remaining 14% represents the residual values of the model (Figure 10A). The experimental values (observed values) and those predicted for the TFC are found in the immediate vicinity of the correlation line, which shows that the model predicts the experimental data very well, fact also proven by the coefficient of determination value $R^2=0.97$ (quadratic model). This indicates that 97% of the experimental data were approximated by the model; the remaining 3% represents the residual values of the model (Figure 10B). The experimental values (observed values) and those predicted for the TAA are found in the immediate vicinity of the correlation line, which confirms that the model predicts the experimental data very well, fact proven by the coefficient of determination value $R^2=0.95$ (quadratic model). This proves that 95% of the experimental data were approximated by the model; the remaining 5% being the residual values of the model (Figure 10C).

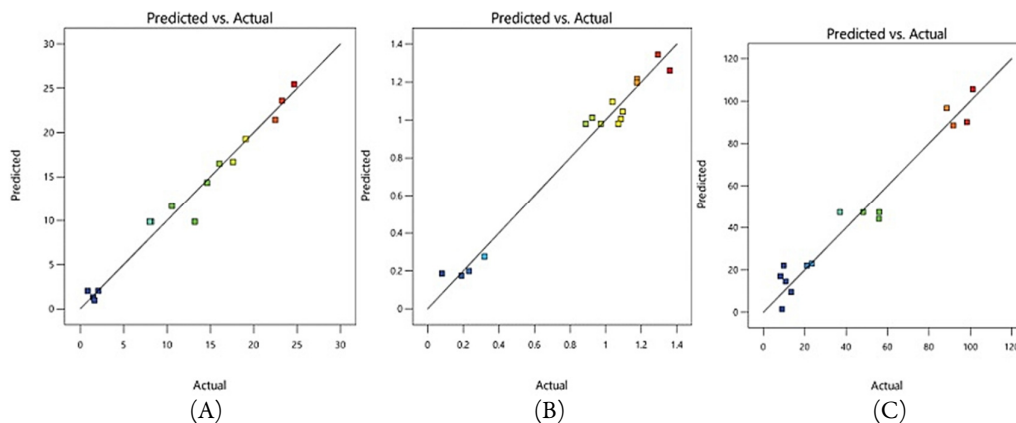


Figure 10. Actual versus predicted values acquired through a second-order polynomial model to analyse the TPC (A), the TFC (B) and the TAA (C)

The study of the extraction parameters effects values on the concentration of the TPC revealed that the EtOH concentration had a high degree of influence, being able to vary between 60-80% when the GAE concentration was between 10-20 mg/g raw material. The time and temperature parameters did not significantly influence the extraction process, the critical values being represented by the temperature of 40 °C and the extraction time of 30 min when a decrease of the TPC was observed (Figure 11A). The study of the effects of the extraction parameters on the concentration of the TFC showed that the EtOH concentration was again very important, being able to vary between 60-80% when the QE concentration is between 1-1.4 mg /g raw material. Moreover, the time and temperature parameters did not significantly influence the extraction process (Figure 11B). The study of the effects of the extraction parameters on the concentration of the TPC showed that EtOH concentration could vary between 80-100% when the concentration of phenolic compounds is between 40-100 mg/g raw material. The time and temperature parameters did not significantly influence the extraction process critical values that were represented by the temperature of 30 °C and the extraction time of 15 min when a decrease of the TPC was detected (Figure 11C).

The central-face cube model for the Box-Behnken central composition revealed the highest concentration value of the TPC (28.43 mg GAE/g raw material) and TFC (1.41 mg QE/g raw material) at a 60% ethanol concentration, 30 °C extraction temperature and a maximum extraction time of 15 min (Figure 12a and Figure 12b). The cube model with the centred faces for the Box-Behnken central composition revealed the highest value of the concentration of the TAA (102.49 mg TE/g raw material) at an 100% ethanol concentration, 50°C extraction temperature and maximum 45 min extraction time (Figure 12c). From the analysis of the experimental values compared to those offered by the model, it was observed that the time variables (for the TFC and the TAA) and temperature (for the TPC) are limiting factors.

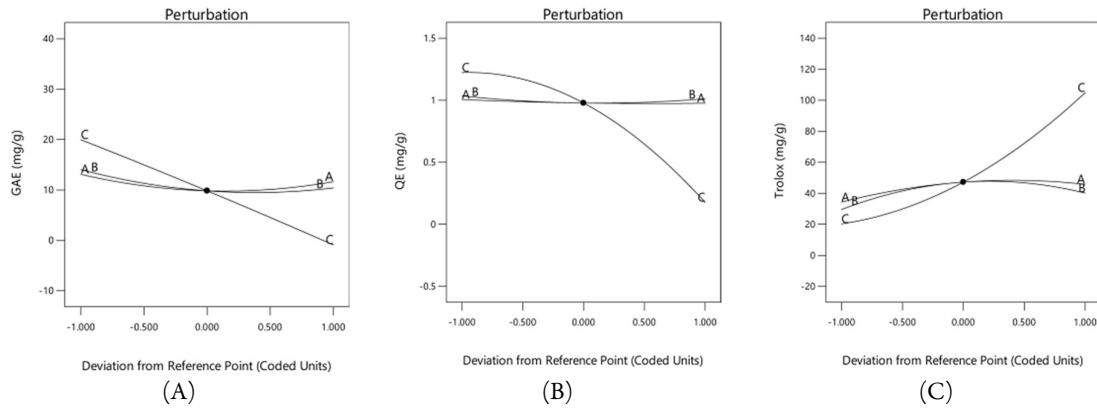


Figure 11. Perturbation graphics acquired using a second-order polynomial model for the TPC (A), the TFC (B) and the TAA (C)

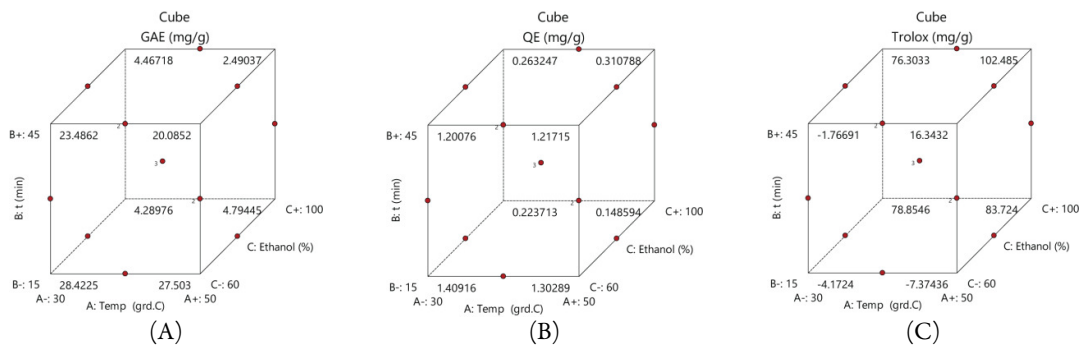


Figure 12. Perturbation graphics acquired using a second-order polynomial model for a the TPC (A), the TFC (B) and the TAA (C)

Conclusions

In our GIS technique research, the validation of the model involved identifying its success rate based on a direct comparison between the predicted and the determined results. The validation of the model through the proposed method displayed a validation rate of 83.5% was obtained which highlights the correctness of the primary databases preparation, the methodology proposed as the basis of the spatial analysis and provides the means for the implementation within the analysed territory, the main purpose being decision-making when planting species adapted to the current climatic and environmental conditions.

The Response Surface Methodology was employed to study the extraction parameters effect: temperature (30-50 °C), time (15-45 min.) and EtOH concentration (60-80%) on the BACs from the cornelian cherry fruits extract. As such, the flavonoids and polyphenols concentrations and the antioxidant activity values were determined. The highest values for the TPC and the TFC were obtained at 60% EtOH concentration and different values for the temperature and time. The most relevant variable was the EtOH concentration, the obtained results suggesting that the experimental data were well approximated by the central-face cube model (86% for the TPC, 97% for the TFC and 95% the TAA).

On the territory of Romania, the beneficiaries of our study can be the territorial administrative units or NGOs regarding reforestation deficient areas in *C. mas*. In addition, the growth of the area of this plant may attract the interest of the food industry in diversifying different ingredients made from this variety. The results of the study create also the premises of further research for obtaining a functional food based on *C. mas* fruits' extract.

Authors' Contributions

Conceptualization EIM, LM; Data curation LM, CV; Formal analysis EIM, SR, GC; Funding acquisition LM; Investigation EIM, SR, GC; Methodology SR, GC; Project administration LM, CV; Resources LM; Software SR, GC; Supervision LM, CV; Validation LM, CV; Visualization LM; Writing - original draft EIM, LM; Writing - review and editing LM, CV. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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