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Optimization of Total Monomeric Anthocyanin (TMA) and Total Phenolic Content (TPC) Extractions from Red Cabbage (*Brassica oleracea* var. *capitata* f. *rubra*): Response Surface Methodology versus Artificial Neural Network

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Abstract:
The aim of this study was to investigate the influence of solvent type, ultrasonic frequency, extraction time and temperature on the total phenolic content (TPC) and total monomeric anthocyanin (TMA) extraction from red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*) using the response surface methodology (RSM) and artificial neural network. The red cabbage has been used as TPC and TMA sources due to its low cost and highly availability during all the year. The experimental data for the extraction of TPC and TMA were fitted to second-order polynomial models with higher regression coefficients than 0.902. The optimal conditions (in dry matter) for highest TPC extraction (7,049.5 mg gallic acid equivalent/kg) are: methanol as solvent, 3.60 kHz ultrasonic frequency at 67.6 °C for 59.6 min, while for TMA optimal extraction (0.3 mg/g) 2-propanol was used as solvent, 45 kHz ultrasonic frequency at 69.2 °C for 20.80 min. The artificial neural network (ANN) is better than RSM to predict the TPC and TMA extraction from red cabbage.

Keywords: red cabbage, total monomeric anthocyanin, total phenol, extraction, optimization
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1 Introduction

Currently, many fruits and vegetables are not used only in food industry for the human consumption; they are used in the pharmaceutical industry as an alternative to classic drugs. Red cabbage (*Brassica oleracea* L. var. *capitata f. rubra*) belongs to the family of *Brassicaceae*, and it originates from the Mediterranean region and South-Western Europe, nowadays being grown in various regions all over the world [1]. The studies conducted on the influence of *Brassica* species on human health have proved their capacity to prevent some type of cancer and cardiovascular diseases. The substances responsible for these properties are polyphenols [2], with the anthocyanins being the most abundant class [1]. Anthocyanins, the largest group of natural pigments are associated with the colour of fruits and plants, and are present in nature mainly in the form of heterosides [3, 4].

Anthocyanidins are linked to one or more glycosidic units, being a glycosylated polyhydroxy- and polymethoxy derivates of 2-phenylbenzopyrylium salts (flavylium). The glycosidic units may be linked to the anthocyanidins by α or β linkage, and always in position 3 of the aglycon (Figure 1). When additional sugars are present in the anthocyanin molecule, they are linked to positions 5 and 7, and less frequently to 3 and 5. The sugars encountered in anthocyanins can be hexoses (glucose and galactose) and pentoses (xylose, arabinose) [4]. Anthocyanin molecules are unstable and easily degraded [5] by temperature, pH, light, oxygen, solvents, metallic ions, ascorbic acid, sulphite and enzymes [3].

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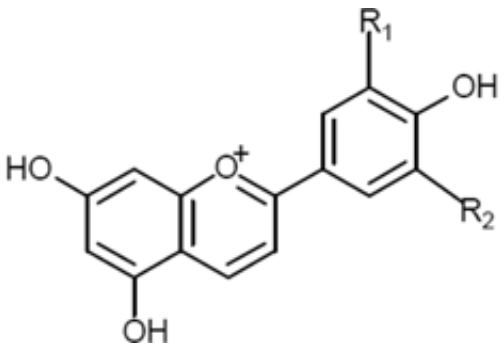

	R_1	R_2
Pelargonidin	H	H
Cyanidin	OH	H
Delphinidin	OH	OH
Paeonidin	OCH_3	H
Petunidin	OCH_3	OH

Figure 1: Anthocyanidins.

Anthocyanins exert beneficial effects on humans; they exhibit antioxidant, and anti-carcinogenic activity. It is proved that a four-week anthocyanin intake decreases myopia [6], apoptosis, and diabetes and obesity symptoms [7].

One of the most suitable tools for studying the influence of various input variables simultaneously to an output response is the response surface methodology (RSM). RSM, originally described by Box & Behnken [8], enables to reduce the number of experimental trials necessary to evaluate the multiple parameters involved in a single process and their interactions need less time and labour. RSM is a collection of statistical and mathematical techniques that have been successfully used at developing and improving processes [9], being widely applied to optimize food industry procedures [10, 11].

Artificial neural networks (ANNs) are an analytical tool based on the structure of biological neurons, and it has been widely used in several fields, including food industry. Unlike other analytical methods, where prior knowledge of relationships among process parameters is required, ANN draws on previously gathered information and utilizes this when analysing new data input [12, 13]. It is particularly useful in managing uncertainties and non-linear data relationships [14]. In food industry, the ANN has been applied to predict the texture characteristics of food [14], sensory attributes of noodles [15], shelf life of soya milk [16] and colour changes of osmotic dehydrated kiwifruit [17].

The aim of this study was to model and optimize the extraction conditions (solvent type, ultrasonic frequency, extraction time and temperature) of total monomer anthocyanins (TMA) and total phenolic content (TPC) from red cabbage using response surface methodology and artificial neural network. The prediction capabilities of the methods (RSM and ANN) were compared using their regression coefficients (R^2) and the absolute average deviation (ADD).

2 Materials and methods

2.1 Sample preparation

Red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*) was purchased from a local producer from Suceava (47°39′5″N 26°15′20″E), Romania in September 2013. The cabbage was cut into small pieces (2 mm particle dimensions), and dried at 40 °C into an oven (Universal Oven U, Memmert, Germany) till constant weight. The powder (75 µm particle diameter) was kept at –20 °C until the extraction process.

Culture condition: Rooted shoots with three to five leaves were transplanted to pots containing soil for acclimation and cultured in a growth chamber with high relative humidity (80 %) for 3–4 weeks before being moved to the field for further growth. The cabbage plants have been spaced about 30–66 cm from one another. They will need watering often but are otherwise low maintenance plants. The red cabbage has been harvested in September 2013. Each cabbage had around 1 kg weight.

2.2 Extraction procedure

To prepare the mixture of the extraction process, 0.5 g of red cabbage powder was mixed with 50 ml of solvent (methanol, ethanol and 2-propanol, respectively). The sample was placed into an ultrasonic bath at different temperatures (50, 60 and 70 °C), for different times (10, 20, 30, 40, 50 and 60 min) at different ultrasonic frequencies (0, 22.5 and 45 kHz).

2.3 Total monomeric anthocyanin determination

The total monomeric anthocyanin was determined using a spectrophotometric method developed by Rabino & Mancinelli [18]. The UV-VIS spectrophotometer (Ocean Optics HR4000, USA) was used for spectral measurements at 657 nm and 530 nm. The net absorbance (A_{net}) was calculated using the net equation:

$$A_{net} = A_{530} - 0.25A_{657}$$

The monomeric anthocyanin content, dry matter, was calculated based on cyanindin-3-glycoside (Cyd-3-glu) which has a molecular weight of 449.1 and extinction coefficient of 29 600 [18].

$$TMA\left(\frac{\text{mg}}{\text{g}}\right) = \frac{A_{net}}{29600} \cdot MW \cdot DF \cdot \frac{V}{\text{Wt}} \quad (1)$$

where MW = 449.1 $M^{-1} \cdot cm^{-1}$, DF – dilution factor, V – total volume (ml), Wt – sample weight (g).

2.4 Total phenolic content

The TPC determinations of extracts (in methanol, ethanol and 2-propanol, respectively) were measured colorimetrically using the Folin-Ciocalteu (FC) method [19, 20]. The raw extract obtained was diluted with a dilution factor of 200. Then, 1.0 ml of the extract aliquot in triplicates was transferred into a test tube and then mixed thoroughly with 5.0 ml of FC reagent priory diluted 1:10 with distilled water. After shaking for 3 min, 4.0 ml of sodium carbonate (7.5 %, w/v) was added to and mixed thoroughly. The mixtures were then allowed to stand for 30 min in the dark before its absorbance was measured in a single beam UV–Vis spectrophotometer (Ocean optics, USA) at 765 nm [21] against the blank of methanol or ethanol pure solvents. The TPC values were expressed in mg gallic acid equivalent (GAE)/kg dry matter.

2.5 Response surface methodology (RSM)

The experiment was conducted into three factor full factorial experiment for each solvent used (methanol, ethanol and 2-propanol). Each parameter (time, temperature and ultrasonic frequency) had at least 3 levels, as follows: time – 3 levels (20, 40 and 60 min respectively), temperature – 3 levels (50, 60 and 70 °C) and ultrasonic frequency – 3 levels (0, 22.5 and 45 kHz). The full factorial design was designed and made using Design expert 9.0 (trial version) to cover the range of investigated ultrasonic treatment time, ultrasonic frequency and temperature. Ultrasonic frequency, time and temperature were chosen as independent variables and the total monomeric anthocyanins (TMA) and total phenolic content (TPC) were responses of the design. The yields of extractions were tested using three types of solvents (methanol, ethanol and 2-propanol).

The model used to predict the evolution of the extraction efficiency was a second-order (quadratic) polynomial response surface model which can be applied to fit the experimental results obtained by Box–Behnken design. The second-order (quadratic) polynomial response surface model which describes the relationship between the experimental results is:

$$y = b_0 + \sum_{i=1}^{n} (b_i x_i) + \sum_{i=1}^{n} \left(b_{ii} x_{ii}^2\right) + \sum_{ij=1}^{n} \left(b_{ij} x_i x_j\right) \quad (2)$$

where: y is the predicted response (TMA or TPC), x_i stands for the coded levels of the design variable (time, temperature and ultrasonic frequency – Table 1), b_0 is a constant, b_i – linear effects, b_{ii} – quadratic effects and b_{ij} – interaction effects.

Table 1: Levels in full factorial experiments for TMA and TPC from red cabbage.

Factor	–1	0	1
Ultrasonic time (min)	20	40	60
Ultrasonic frequency (kHz)	0	22.5	45
Temperature (°C)	50	60	70

The optimization of parameters simultaneously is very important to predict the extraction of TMA and TPC. The desirability function approach is used to optimize the multiple characteristics concurrently [22].

2.6 Artificial neural network (ANN)

The data used for ANN modelling were used for RSM too. The ANN modelling was made up using the NeuroSolutions 6.0 trial version (NeuroDimension Inc., Gainesville, FL, USA). The software for ANN has an advanced neural networks wizard known as the Neural Builder providing more powerful nonlinear analysis methods to build the network "architecture". The network "architecture" is a representation by a graph where the input variables are organized in layers, while the weights modulating the combination of non-linear functions are represented as lines connecting units in different layers. The nonlinear analysis method chosen in this software is known as the multilayer perceptron (MLP) where it belongs to the supervised learning neural network, being very important in engineering applications. It also provides flexibility and complexity to approximate nonlinear functions to any desired accuracy by changing the number of layers and the number of neurons in each layer [17, 23, 24]. The performance of the network is explicitly measured based on the mean squared error (MSE), defined as the difference of the output of ANN and a pre-specified external desired signal.

The exact MLP network architecture of this study has an input layer of three neurons, one hidden layer of twelve neurons and an output layer of two neurons as illustrated in Figure 2. The input layer is comprised of three neurons due to the three input variables: ultrasonic frequency, extraction temperature and time. The output layer consists of one neuron representing the TPC and TMA. The lines connecting the input neurons and hidden neurons represent the weights. The hidden neurons sum the corresponding weighted inputs denoted in Figure 2. Each hidden neurons passes its weighted sum through a hyperbolic tangent function as the hidden layer transfer function. In this study, 12 neurons in the hidden layer were determined from the lowest value of mean squared errors (MSE) of 0.020. The choice of one hidden layer is usually sufficient in approximating the continuous nonlinear function as more hidden layers may cause over-fitting [23–26]. Besides the number of neurons in hidden layers, the momentum and learning rate are two important settings for this MLP network (Figure 2). The recommended momentum and learning rate values of 0.7 and 0.1 were used in this study to calculate the weights [27].The response of predicted TPC obtained from RSM and ANN was then compared using the absolute average deviation (ADD) given in eq. (3) [28]:

$$ADD = \frac{\sum_{i=1}^{P} \left(\left| Y_{i,exp} - Y_{i,cal} \right| \right) / Y_{i,exp}}{P} x100 \tag{3}$$

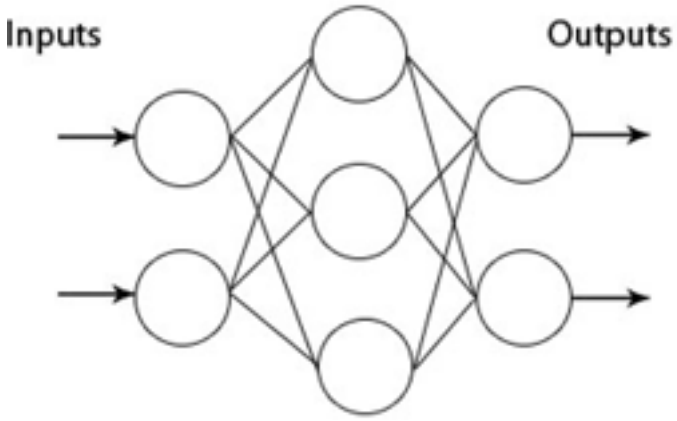

Figure 2: Schematic of multilayer perceptron (MLP) [20, 23–25, 27].

where $Y_{i,exp}$ and $Y_{i,cal}$ are the experimental and calculated responses, and P is the number of the experimental run. Absolute average deviation (AAD) in this study is a measure of how much the predicted data of models deviate from the experimental data [26].

3 Results and discussion

3.1 Extraction time and temperature influence

Extraction time and temperature is an important parameter which should be optimized in order to minimize energy cost of the process. Kinetics of TMA and TPC extraction from red cabbage are shown in Figure 3 and Figure 4. The extraction yield of the two parameters increased with the temperature, the same observation being made by D'Allesandro et al. [29].

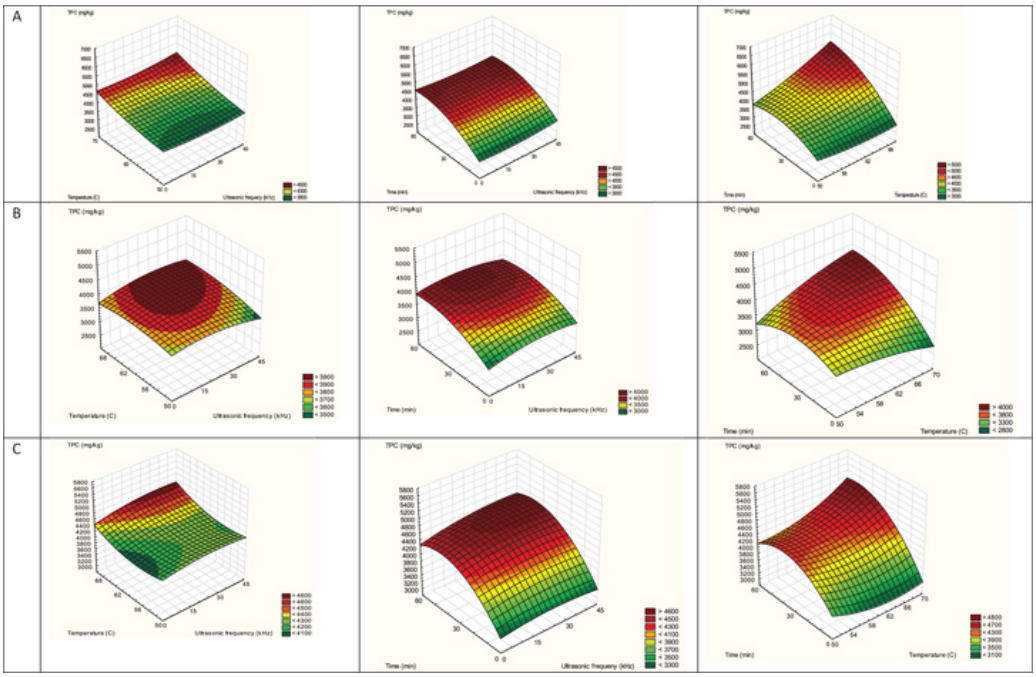

Figure 3: The evolution of TPC in function of temperature, time and ultrasonic frequency using methanol (A), ethanol (B) and 2-propanol (C) as solvents.

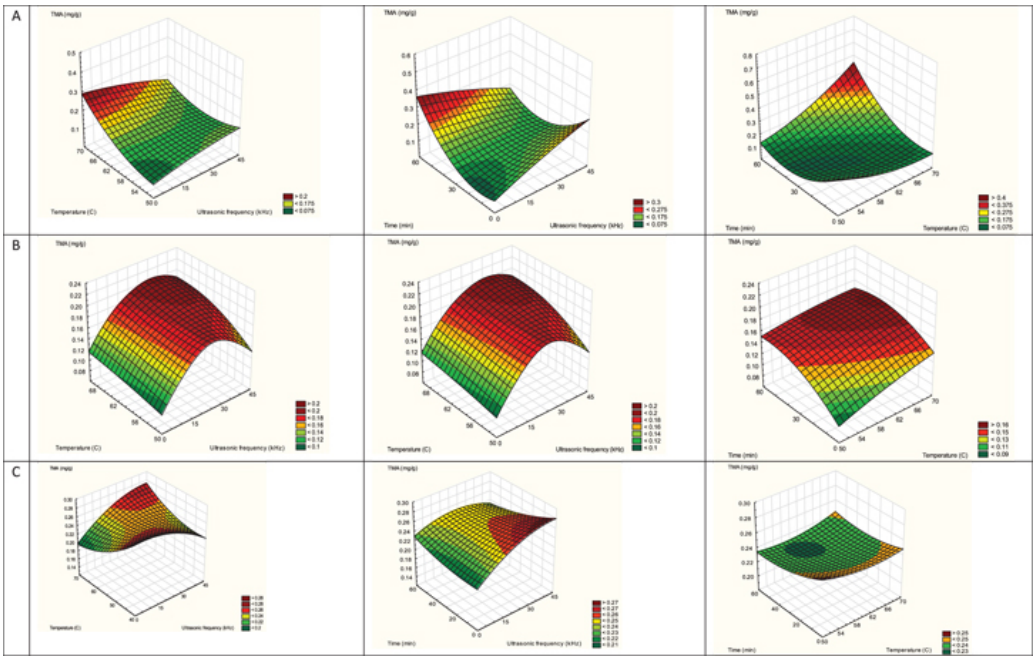

Figure 4: The evolution of TMA in function of temperature, time and ultrasonic frequency using methanol (A), ethanol (B) and 2-propanol (C) as solvents.

The temperature influences positively the extraction of TMA and TPC. The increase of temperature extraction from 50 °C to 70 °C leads to an increase of extraction efficiency by 16.5 % in the case of TMA and 22.6 % in

the case of TPC, respectively. Arapitsas & Turner [1] observed that extraction temperature and time influence positively the extraction efficiency of anthocyanins.

The positive influence of temperature can be explained by the fact that polyphenols increase their solubility in the solvent with the temperature increase; at higher temperature the diffusivities of the extracted molecules increases and in consequence the mass transfer [29–31]. The increase of temperature may break the phenolic matrix bonds and influence the membrane structure of plant cells and therefore facilitates the extraction process [29–31]. However, the use of very high temperatures in polyphenols extraction can lead to loss of solvent and promote polyphenols oxidation caused by hydrolysis or internal redox reactions, as it was reported by Rostango et al. [30].

3.2 Solvent-type influence

Different types of solvents are generally used for the anthocyanin/polyphenol extraction such as: methanol, ethanol, acetone, formic acid, 2-propanol etc. [32].. Over the last years, methanol was replaced by ethanol due to the high human toxicity of the former [33]. Alcoholic solvents give quite high yield of total extract [29].

It can be observed in Figure 3 and Figure 4 that methanol is a suitable solvent in the case of TPC, while 2-propanol for the TMA. In the case of TMA, 2-propanol had the highest extraction concentration even when the temperature is 50, 60 or 70 °C or the ultrasound frequency is 0, 22.5 and 45 kHz. The highest concentration of TMA observed was of 0.291 mg/g using 2-propanol as solvent, being 33.4 % higher than in the case of ethanol as solvent (0.218 mg/g) and with 25.3 % higher than in the case of methanol as solvent (0.232 mg/g). In the case of TPC the highest concentration was of 6,788.4 mg/kg using methanol as solvent with 20.03 % higher than in the case of 2-propanol as solvent (5,655.2 mg/kg) and with 26.5 % higher than in the case of ethanol as solvent (5,364.2 mg/kg), respectively.

The differences in the red cabbage extract yields in the present study might comparing with other studies [34] be explained by the different availability of extractable components, resulting from the varied chemical composition of plants. The amount of antioxidant components that can be extracted from red cabbage is mainly affected by the vigour of the extraction procedure, which may probably vary from sample to sample. Amongst other contributing factors, efficiency of the extracting solvent to dissolve endogenous compounds might also be very important [35–37].

The affinity of polyphenols for methanol as against ethanol and 2-propanol with reference to solubilisation of materials in red cabbage may be related to the dielectric constant (methanol has a higher dielectric constant than ethanol and 2-propanol) [38]. In the case of TMA the affinity could be related to a low dipole moment of the 2-propanol in comparison with ethanol and methanol. The extraction of TMA may be influenced by the surface tension of the solvent as 2-propanol (23.3 mJ/cm^2) has a higher surface tension than methanol (22.1 mJ/cm^2) and ethanol (22.0 mJ/cm^2) [39].

3.3 Influence of ultrasonic frequency

Figure 3 and Figure 4 present TPC and TMA extraction, depending on ultrasonic frequency. It can be observed that frequency had a positively significant influence on the TMA and TPC extraction. The extraction efficiency increased with the ultrasonic frequency. The highest TPC extraction was of 6,788 mg/kg at 45 kHz with 18.4 % higher than in the case of 22.5 kHz (5,737.5 mg/kg) and 25.7 % higher than in the case of 0 kHz (5,396.6 mg/kg), respectively. In the case of TMA, the highest concentration was of 0.291 mg/g in the case of 45 kHz with 1.7 % higher than in the case of 22.5 kHz (0.285 mg/g) and 9.9 % higher than in the case of 0 kHz (0.262 mg/g).

During sonication, the cavitation process provokes swelling of cells, solvent uptake and enlargement of the pores of the cell walls, which allow higher diffusivity across the cell walls. Enhanced extraction yields allow higher diffusivity across the cell walls. Enhanced extraction yields obtained at ultrasound assistance could be attributed to the fact the sonication could incite the breakdown of cell walls and facilitate the washing out of the cell content [29].

3.4 Suitable solvent, time, temperature and ultrasonic frequency for TPC and TMA extraction

The best conditions for TPC (6,788.4 mg/kg) and TMA (0.291 mg/g) extraction are: methanol, 60 min, 70 °C and 45 kHz for and 2-propanol, 10 min, 70 °C and 45 kHz, respectively. The antioxidant activity of red cabbage is strongly dependent on the solvent due to the different antioxidant potential of compounds with different polarity [38, 39] and Folin Ciocalteu assay gives a crude estimate of the total phenolic compounds present in

an extract; it is not specific to polyphenols, but many interfering compounds may react with reagent giving elevated apparent phenolic concentrations [40, 42, 43].

3.5 Response surface methodology (RSM)

The RSM methodology has been made up using the Box–Behnken design with 15 experiments (presented in Table 2), with no blocks.

Table 2: Box-Behnken design with coded values for the RSM.

Run no.	Ultrasonic frequency, U	Temperature, T	Time, t
1	0	0	0
2	–1	–1	0
3	1	–1	0
4	–1	1	0
5	1	1	0
6	–1	0	–1
7	1	0	–1
8	0	0	0
9	–1	0	1
10	1	0	1
11	0	–1	–1
12	0	1	–1
13	0	–1	1
14	0	1	1
15	0	0	0

3.5.1 TPC

The TPC data extracted using the three solvents (methanol, ethanol and 2-propanol) in function of three parameters (ultrasonic frequency, time and temperature) were fitted to quadratic equation using response surface analysis. The equations for TP (TP_m – total phenolic content using methanol as solvent, TP_e – total phenolic content using ethanol as solvent, TP_p total phenolic content using 2-propanol as solvent) are presented in the eqs (4)–(6):

$$TP_m = 3800.00 - 12.73 \cdot U + 233.69 \cdot T - 52.50 \cdot t + 501.34 \cdot U^2 + 207.77 \cdot T^2 + 146.56 \cdot t^2 + 67.47 \cdot U \cdot T - 269.88 \cdot U \cdot t + 518.76 \cdot T \cdot t \quad (4)$$

$$TP_e = 2947.45 + 38.86 \cdot U + 57.19 \cdot T - 17.65 \cdot t + 363.27 \cdot U^2 + 314.57 \cdot T^2 + 623.32 \cdot t^2 + 37.98 \cdot U \cdot T - 62.56 \cdot U \cdot t + 475.87 \cdot T \cdot t \quad (5)$$

$$TP_p = 3973.37 + 177.84 \cdot U + 38.43 \cdot T + 59.43 \cdot t + 339.59 \cdot U^2 + 161.75 \cdot T^2 + 50.04 \cdot t^2 - 263.63 \cdot U \cdot T + 216.26 \cdot U \cdot t + 289.54 \cdot T \cdot t \quad (6)$$

The sum of squares, mean square, F – ratio and the coefficient of regression for each model are shown in the Table 3. All the models proposed are significant ($p < 0.05$). The coefficients of regressions (R^2) obtained for the above quadratic equation indicated that the variation of total phenol content yield can be explained by independent variables of ultrasonic frequency (U), temperature (T) and time (t) of extraction. The regression analysis revealed that ultrasonic frequency had significant positively linear effects on total phenol content irrespective of the solvent type, while the temperature had significant negatively linear effects on total phenol content in the case of methanol as extraction solvent, whereas in the case of ethanol and 2-propanol it had not been observed negatively linear effects on total phenol content. The interactions between one parameter with another one are generally insignificant ($p > 0.05$), only the interaction between time and temperature in the case of ethanol as extraction solvent is a significant one ($p < 0.05$).

Table 3: Sum of square, mean square, F-value, R^2 and ADD values of response surface methodology.

Solvent	Parameter	Sum of squares	Mean square	F-value	R^2	ADD
2-propanol	TPC	$4.494 \cdot 10^5$	$1.650 \cdot 10^5$	4.86*	0.905	4.067
Methanol	TPC	$1.070 \cdot 10^6$	$3.562 \cdot 10^5$	3.59*	0.956	7.004
Ethanol	TPC	$2.026 \cdot 10^6$	$6.754 \cdot 10^5$	5.41**	0.926	2.940
2-propanol	TMA	0.002	0.004	4.57*	0.922	6.691
Methanol	TMA	0.007	0.003	4.26**	0.921	9.764
Ethanol	TMA	0.008	0.003	3.95*	0.902	7.960

* $-P < 0.05$
** $-P < 0.01$

The optimization of ultrasonic frequency, extraction time and temperature and solvent were made so as the TPC extraction to be maxim. Using the RSM methodology for optimization it seems that methanol is the suitable solvent and if the ultrasonic frequency is of 3.60 kHz, the temperature 67.6 °C and the time 59.6 min, the TPC will be of 7,049.5 mg gallic acid equivalent (GAE)/kg (d = 0.873).

3.5.2 TMA

The TMA data obtained using the three solvents (methanol, ethanol and 2-propanol) in function of three parameters (ultrasonic frequency, time and temperature) were fitted to quadratic equation using response surface analysis. The equations for the TMA (TMA_m – total monomeric anthocyanin using methanol as solvent, TMA_e – total monomeric anthocyanin using ethanol as solvent, TMA_p total monomeric anthocyanin using 2-propanol as solvent) are presented in the eqs (7)–(9):

$$TMA_m = 0.15 + 0.039 \cdot U + 0.012 \cdot T + 0.007 \cdot t - 0.033 \cdot U^2 + 0.029 \cdot T^2 - 0.002 \cdot t^2 + 0.028 \cdot U \cdot T + 0.004 \cdot U \cdot t + 0.023 \cdot T \cdot t \quad (7)$$

$$TMA_e = 0.19 + 0.035 \cdot U + 0.006 \cdot T + 0.001 \cdot t - 0.045 \cdot U^2 + 0.003 \cdot T^2 + 0.003 \cdot t^2 + 0.012 \cdot U \cdot T - 0.002 \cdot U \cdot t + 0.002 \cdot T \cdot t \quad (8)$$

$$TMA_p = 0.22 + 0.012 \cdot U - 0.008 \cdot T + 0.003 \cdot t + 0.008 \cdot U^2 + 0.015 \cdot T^2 + 0.008 \cdot t^2 + 0.022 \cdot U \cdot T - 0.007 \cdot U \cdot t + 0.003 \cdot T \cdot t \quad (9)$$

The sum of squares, mean square, F – ratio and the coefficient of regression for each model are presented in the Table 3. All the models proposed are significant ($p < 0.05$). The coefficients of regressions (R^2) obtained for the above quadratic equation indicated that the variation of total phenol content yield can be explained by independent variables of ultrasonic frequency (U), temperature (T) and time (t) of extraction. In the TMA have been observed positively significant linear effect of the three parameters applied (ultrasonic frequency, time and temperature) for ethanol and methanol. In the case of 2-propanol, the ultrasonic frequency and the extraction time have a positively significant linear effect on TMA, while time and temperature have a negatively significant linear effect. The interaction between ultrasonic frequency and time and ultrasonic frequency with temperature have significant effect ($p < 0.05$) in the case of 2-propanol, while the rest of interaction between each one parameter for the rest of possibilities does not have significant effects ($p > 0.05$).

The optimization of ultrasonic frequency, extraction time and temperature and solvent were made so as the TMA extraction to be maximum. Using the RSM methodology for optimization it seems that 2-propanol is the suitable solvent and if the ultrasonic frequency is of 45 kHz, the temperature 69.2 °C and the time 20.80 min, the TPC will be of 0.30 mg expressed as cyanindin-3-glycoside /g (d = 0.814).

3.6 Artificial network prediction for TPC and TMA

TMA and TPC prediction using the ANN was limited to the selection of a suitable number of neurons in the hidden layer as the number of neurons for input and output layers were already defined from the experimental design. The ANN design, for TMA and TPC, was made using 3 input layers (ultrasonic frequency, temperature and time), the number of neurons in the hidden layer was determined after having run several networks

iteratively and after having observed the minimum value in the mean squared errors (MSE) and regression coefficients.

The Table 4 presents the regression coefficients and ADD values for the ANN prediction of TPC and TMA, for the three solvents used. The AADs (Table 4) ranged between 1.817 and 6.843, much lower than the values observed in the case of response surface analysis (Table 4). The same evolution was observed in the case of correlation coefficients too. Keeping into account the regression coefficients and ADD values it seems that the ANN is a better predictor as compared to the response surface analysis. Other previous studies where a comparison was drawn on the usefulness of ANN and response surface analysis have led to results similar to those of this paper. Bas & Boyaci [30] in their study on biochemical reaction observed that the ANN is better than the response surface methodology to model nonlinear data. Cheok et al. [26] observed that the ANN is better than the response surface methodology to model nonlinear data of TPC extracted from *Garcinia mangostana* Linn. The same observation was observed in the case of extraction of epigallocatechin-3-galate [44], flavonoids extraction from *Artemisia argyi* [45] and antioxidants from spray dried pomegranate juice [46].

Table 4: Regression coefficients and ADD values for ANN prediction of TPC and TMA.

MLP	Solvent	R^2	ADD
TPC	2-propanol	0.916	3.816
	Ethanol	0.971	1.817
	Methanol	0.962	6.843
TMA	2-propanol	0.925	3.953
	Ethanol	0.955	4.916
	Methanol	0.972	5.821

The importance of the independent variables of ultrasonic frequency, temperature and time for the ANN prediction of TMA and TPC are presented in Table 5. In the case of TMA the ultrasonic frequency and the temperature have the highest importance, while in the case of TPC for each solvent there is a different variable which has the highest importance.

Table 5: Independent variable importance of the ANN prediction of TMA and TPC extraction.

	Ultrasonic frequency	Temperature	Time
TMA			
Methanol	0.578	0.225	0.196
Ethanol	0.428	0.435	0.137
2-propanol	0.428	0.435	0.137
TPC			
Methanol	0.156	0.440	0.404
Ethanol	0.360	0.320	0.320
2-propanol	0.325	0.095	0.579

4 Conclusions

The ultrasonic frequency, extraction time and temperature had an important influence on the extraction efficiency of TMA and TPC from red cabbage. The suitable solvents for TMA and TPC extraction are 2-propanol and methanol respectively. The extraction efficiency is increased with the temperature increasing therefore 70 °C is the suitable temperature. The ultrasonic frequency increases the extraction, 45 kHz being the best frequency. The response surface methodology is a useful mathematical tool to optimize the extraction process, while the artificial neural network is a better method for data prediction.

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