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The Influence of Water Extraction Parameters in Subcritical Conditions and the Shape of the Reactor on the Quality of Extracts Obtained from Norway Maple (*Acer platanoides* L.)

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Abstract: The Box–Behnken experimental design was used to investigate the effect of subcritical water extraction parameters such as temperature, process duration, and extractor shape on the extract composition and antioxidant activity of Norway maple (*Acer platanoides* L.) bark extracts. Spectrophotometric (UV-Vis) techniques were employed to evaluate the total polyphenols (TPC) and flavonoids (TFC). The DPPH radical scavenging method was used to evaluate the antioxidant activity of the extracts. The yield of the process was evaluated through the utilization of response surface methodology (RSM). The total polyphenol and flavonoid contents, together with antioxidant activity, are highly dependent on water temperature. The influence of changes in the process duration and the shape of the pressure cell was not observed. A temperature increase from 110 °C to 170 °C caused a 8.9-fold increase in the polyphenol content, 7.2-fold increase in the flavonoid content, and 12.6-fold increase in the antioxidant activity. The highest values for polyphenols, flavonoids, and antioxidant activity occurred at a temperature of 170 °C, which is the upper limit of the temperature variability range for these studies. This study demonstrates the importance of the appropriate selection of extraction parameters in order to obtain the desired chemical composition of the extract.

Keywords: maple; *Acer platanoides* L.; subcritical water; Box–Behnken; extraction; polyphenols; flavonoids

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1. Introduction

The bark of trees is a plant material, processed mainly into thermal energy and chemical by-products; it is also a source of valuable biologically active substances. Polyphenols are metabolites that exist in plants [1,2]. They are part of biologically active substances, and various enzymes affecting metabolic reactions influence their production [3]. The biochemical and morphological regulatory patterns of plants are integrated within the metabolism of these compounds [4]. Defence mechanisms in the plants are driven by phenolic compounds [5,6]. Polyphenols are recognized as important food ingredients, with health-promoting benefits [7]. Maple sap is consumed as a tonic with health-beneficial properties [8]. The antioxidant activity of sugar maple leaves is correlated with polyphenols and with harvesting time; minimum phenolic (105.67 mg GAE/g dry mass) (GAE—gallic acid equivalent) and flavonoid content (3.27 ± 0.26 mg CTE/g dry mass) (CTE—catechin equivalent) were obtained by extraction from fall leaves [9]. The content of antioxidant components in extracts obtained using microwave-assisted extraction for sugar maple ranged as follows: total phenols: 35.77 to 136.55 mgGAE/g DM (DM—dry mass); total flavonoids: 10.51 to 47.33 mg CTE/g DM; condensed tannins: 5.33 to 127.33 mg CTE/g DM; and extractable tannins: 32.21 to 110.35 mg GAE/g DM [10]. Phenolics are metabolites

produced by plants, and concentrations of phenolic compounds can vary for different parts of the plant [11]. When examining harvestable plant tissues and organs, it was found that the polyphenol content in plants varied for different elements [12]. The concentration of biologically active substances for red maple was found to be in the following ascending order: stem bark, the bark of branches, and twigs [7]. The content of polyphenols in walnut was found to be in the following order: main root, buds, leaves, and bark [13]. Sugar maple bark extracts obtained using acetone as a solvent contained mainly p-hydroxy benzoic acid (8950.5 μg/g extract), gallic acid (5261 μg/g extract), and salicylic acid (572.38 μg/g extract). The high polyphenol content (292.67 mg GAE/g dry mass) was correlated with high antioxidant activity (IC50 values of 1.77 and 4.14 μg/mL) [14]. In traditional medicine, the bark of maple has been used in the treatment of ailments like eye diseases and back pain and as a diuretic [15,16]. The extracts obtained from the bark of maple contain phenolic compounds like gallic acid derivatives and flavonoids such as quercetin glycosides, rutin, and kaempferol [7,17,18]. Extracts obtained from 250 g of sugar and red maple bark with a moisture content of 5.6% and 9.5%, ground to particle sizes from 250 to 500 μm, separately extracted using 2.5 L of water as a solvent, for 1 h duration and under conditions of 90 °C achieved the following: total polyphenol content: 19.04 and 40.12 g GAE/100 g DE; total flavonoid content: 1.46 and 1.58 g QE/100 g DE; antioxidant activity (ABTS assay): 45.20 and 128.71 mmol TE/100 g DE (TE—Trolox equivalent) [19].

Water extraction under subcritical conditions is considered an environmentally friendly separation technique for bioactive compounds from plant materials. It should also be noted that these techniques can be scaled to industrial size [20–22]. The unique properties of water under subcritical conditions include high dielectric constant, high boiling point, and high polarity [23]. The electric permittivity of the water falls as the temperature rises, but the diffusivity rises and the viscosity and surface tension both decrease. In consequence, materials that are highly polar and easily soluble in water within normal conditions can be separated more effectively in low temperatures than low-polar molecules, which need a low polar medium, present in higher temperatures [1,24,25]. A rise in water temperature enhances the diffusion rate and the kinetics of desorption and leads to an increase in compound dissociation. The quality of contact between plant material and solvent can be highly improved by viscosity and surface tension reduction at higher process temperatures. Taking into account the above-mentioned changes in water properties associated with temperature increase, the process rate and efficiency can be improved by increasing the temperature of the process.

Studies on the subcritical extraction method show that the extracts produced using this method have better antioxidant capacities. They also highlight a strong correlation between process temperature and antioxidant activity [25–30].

Knowing the impact of process variables such as temperature, reactor design, and process time on the quality of extracts is crucial for designing optimal extraction devices, allowing reduced production costs and increased process efficiency.

The literature describes the bioactivity of individual parts for sugar and red maple, such as the main root, buds, leaves, bark, and petioles, but does not contain information on Norway maple, especially in terms of the impact of extraction cell construction and process parameters on the process efficiency.

2. Materials and Methods

2.1. Raw Material

Norway maple (*Acer platanoides* L.) branch bark was harvested for research in the Polish State Forests under supervision of the employees of the Puławy Forest District (location 51°26′02.2″ N, 22°00′09.0″ E). Material for the extraction process was obtained from branches of trees (15 years old). Maple bark was dried by natural convection at an ambient temperature of 20 °C. Then, the bark was ground using a RETSCH SM100 cutting mill with blade speed of 9.4 m/s. A fraction with a size from 0.9 to 1.4 mm was separated using a MULTISERV LPzE-2e laboratory shaker under the following conditions:

separation time of 30 min, frequency of 50 Hz, and vibration amplitude of 2.5 mm. The selected material was further dried to moisture content of 8.09%. The raw material before fragmentation is presented in Figure 1.

Figure 1. Norway maple (*Acer platanoides* L.) bark before fragmentation.

2.2. Reagents

The spectrophotometric assays were conducted using the following chemical reagents: Folin–Ciocalteu reagent (AKTYN, Suchy Las, Poland), sodium carbonate (≥99%, Stanlab, Lublin, Poland), gallic acid (≥98%, Sigma Aldrich, Merck, Germany), 6-hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylic acid (Trolox, >98%, Sigma Aldrich, Merck, Germany), methanol (≥99%, Stanlab, Poland), catechin (≥99.05%, Sigma Aldrich, Merck, Germany), aluminum chloride (>98%, Sigma Aldrich, Merck, Germany), 2,2-diphentyl-1-picrylhydrazyl (DPPH, Sigma Aldrich, Merck, Germany).

2.3. Methods

2.3.1. Design of Experiment

The research was carried out on the basis of an experiment plan generated using the Box–Behnken method in Design-Expert v13. A three-level, three-factor experimental plan was utilized to identify optimal process parameters for *Acer platanoides* L. The three factor levels were characterized as minimum, mean, and maximum examined values of the process parameter. Water temperature (Factor 1), reactor diameter (Factor 2), and process time (Factor 3) served as independent variables, while total polyphenol content (TPC), total flavonoid content (TFC), and antioxidant activity were assumed as dependent variables.

The experiment comprised 17 different combinations, incorporating five center points to assess the pure error. The actual process parameter values for each variable set according to the experiment design are presented in Table 1.

The values obtained in this study were evaluated using analysis of variance ANOVA. The F-test was used to determine the statistical significance of the regression coefficients, with a p-value of less than 0.05 being deemed significant. The model was validated by comparison of the experimental and predicted values.

Table 1. Experimental plan.

Set	A: Process Temperature (°C)	B: Reactor Diameter (mm)	C: Process Time (min)
1	170	10	10
2	170	19.4	5
3	110	19.4	5
4	110	10	10
5	140	10	5
6	140	19.4	10
7	110	19.4	15
8	140	19.4	10
9	110	28.8	10
10	140	28.8	5
11	170	28.8	10
12	140	19.4	10
13	140	28.8	15
14	170	19.4	15
15	140	19.4	10
16	140	19.4	10
17	140	10	15

2.3.2. Preparation of Water Extracts

A Dionex ASE350 automatic extraction device (accelerated solvent extractor) was used to obtain subcritical water extracts. The extraction system was equipped with pressure cells with a volume of 100 mL (diameter 28.8 mm), 45 mL (diameter 19.4 mm), and 12 mL (diameter 10 mm). Fiberglass filters were used in the pressure cells to protect the system. The mass of the raw material for the extraction process was selected to ensure a constant ratio of the raw material weight to the volume of the pressure cell and was as follows: 16.59 g for 100 mL, 7.53 g for 4 5 mL, and 2.00 g for 12 mL. Analytical purity water with a conductivity of 0.09 µS/cm was filled into the pressure cell for the extraction process. Then, a pressure cell filled with water and raw material was heated to temperature according to the experimental plan (DoE—Design of Experiment) and left for the process duration according to the DoE. Then, the extract was dried by water evaporation at a temperature of 40 °C under vacuum conditions. The obtained extracts were stored in the laboratory fridge at a temperature of 2 °C for further analysis.

2.3.3. Results of Chemical Analyses–Total Polyphenol Content

Gallic acid was used as the reference standard for spectrophotometry to determine the total polyphenol content. TPC was determined using the procedure outlined by Sahin et al. [26]. Polyphenol content is presented in units mg(GAE)/100 g DM (dry mass) of raw material. The total polyphenol content was calculated using the following calibration curve.

$$TPC = 0.1075A + 0.0332 \ (R^2 = 0.9982) \quad (1)$$

where TPC—total polyphenol content (10^{-6} g(GAE)/mL), A—absorbance (dim).

2.3.4. Results of Chemical Analyses—Total Flavonoid Content

Catechin was used as the reference standard for spectrophotometry to determine the total flavonoid content. The method outlined by Aryal et al. [31] with a few adjustments [32] was used to measure TFC. The extract sample (1.0 mL) was blended with 1 mL of a methanol-based 2% $AlCl_3 \cdot 6H_2O$ solution. Distilled water was added to the mixture to reach 10 mL. The mixture was incubated at room temperature in the dark for 10 min, and the absorbance was measured at 510 nm. The obtained results are presented in units mg

(CTE)/100 g DM (dry mass) of raw material. The total flavonoid content was calculated using the following calibration curve.

$$TFC = 0.0334A - 0.0093 \ (R^2 = 0.9995) \quad (2)$$

where TFC—total flavonoid content (10^{-6} g(CTE)/mL), A—absorbance (dim).

2.3.5. Results of Chemical Analyses—Antioxidant Activity

The method outlined by Blois [33] with a few adjustments [32] was used to measure antioxidant activity with the DPPH assay application. A 5.8 mL aliquot of freshly prepared $6 \cdot 10^{-5}$ M DPPH radical in methanol was blended with 0.2 mL of extract. Using methanol as a blank, the spectrophotometric absorbance was measured at 516 nm following a 30-min incubation period at room temperature. The measurement of each sample was replicated three times. The obtained results are presented as a Trolox equivalent: 10^{-6} MTE/1 g (dry mass) [32]. The DPPH method was utilized to determine antioxidant activity and the resulting calibration curve was obtained.

$$AC_{DPPH} = 10.279A - 3.0626 \ (R^2 = 0.9993) \quad (3)$$

where AC_{DPPH}—antioxidant activity (10^{-6} MTE/mL), A—absorbance (dim).

3. Results and Discussion

3.1. The Efficiency of the Extraction Process

The efficiency of the extraction process on the bark of Norway maple (*Acer platanoides* L.) was evaluated by comparing the mass of the dry extract to the dry raw material mass. The obtained results varied between 2.02 and 13.53%, depending on the specific experimental settings. The results for each set of Design of Experiments (DoE) are included in Table 2. Table 3 includes the results of analyses of total polyphenol and flavonoid contents, together with antioxidant activities.

Table 2. Efficiency of the extraction process.

Set	A: Process Temperature (°C)	B: Reactor Diameter (mm)	C: Process Time (min)	Extract (Dry Mass) (g)	Extraction Yield (Dry Mass) (%)
1	170	10	10	153.74	7.69
2	170	19.4	5	734.49	9.75
3	110	19.4	5	519.60	6.90
4	110	10	10	40.30	2.02
5	140	10	5	125.04	6.25
6	140	19.4	10	437.40	5.81
7	110	19.4	15	291.28	3.87
8	140	19.4	10	429.24	5.70
9	110	28.8	10	607.77	3.66
10	140	28.8	5	786.26	4.74
11	170	28.8	10	1650.17	9.95
12	140	19.4	10	434.59	5.77
13	140	28.8	15	884.02	5.33
14	170	19.4	15	1018.95	13.53
15	140	19.4	10	444.78	5.91
16	140	19.4	10	420.12	5.58
17	140	10	15	136.42	6.82

Table 3. Results of chemical analyses for total polyphenol and flavonoid contents, together with antioxidant activities.

Set	Polyphenol Content mg (GAE)/100 g (Dry Mass)	Flavonoid Content mg (CTE)/100 g (Dry Mass)	Antioxidant Activity (DPPH) 10^{-6} MTE/1 g (Dry Mass)
1	424	99	4.8
2	732	174	12.53
3	182	49	2.28
4	106	26	1.29
5	428	117	5.78
6	408	103	5.09
7	212	54	2.73
8	374	105	5.2
9	199	53	2.33
10	341	89	3.88
11	761	176	10.85
12	419	106	5.23
13	371	98	3.9
14	943	188	16.24
15	440	111	5.51
16	393	97	3.92
17	489	127	6.52

The lowest extraction yield (2.02%) was achieved for data set number 4, for which the process temperature was 110 °C, which was the lowest value of the temperature variability range. The highest extraction yield (13.53%) was achieved for data set number 14, for which the process temperature was 170 °C, which was the highest value of the temperature variability range. The same relationship was observed in the case of the content of polyphenols and flavonoids as well as antioxidant activity.

3.2. Total Polyphenol Content (TPC)

Depending on the experimental conditions, polyphenol content in the extracts obtained from the bark of Norway maple (*Acer platanoides* L.) ranged from 106 to 943 mg (GAE)/100 g (DM). Figure 2 shows the dependence of polyphenol content in relation to process temperature.

The blue dashed lines in Figures 2–4 indicate 95% confidence intervals of the mean response. The black squares at the ends of the charts indicate the limits of the design space. The red filled circles represent the design (central) points that were used to verify the mathematical model.

Based on the analytical results of the polyphenol content, a multivariable analysis of variance was conducted, and the results indicate that the total polyphenols were dependent only on the process temperature. The linear model represents the relation between process temperature and polyphenol content. An increase in the temperature of the process causes an increase in the content of polyphenols in the extract throughout the entire range of the tested parameter. The influence of the reactor shape and process time changes was not observed. Table 4 presents details of ANOVA analysis.

The model is significant, as indicated by the F-value of 174.92. The likelihood of noise producing an F-value this high is merely 0.01%. *p*-values below 0.05 imply that the model terms are significant. The letter A is a significant model term in this particular case. Considering the pure error, the lack of fit appears not to be significant, as indicated by the 5.23 lack-of-fit F-value. There is a reasonable agreement between the adjusted R^2 (0.9255) and the predicted R^2 (0.9046). Adeq. precision, defined as signal-to-noise ratio, with a value of 30.2539, demonstrates an appropriate signal. The resulting model is statistically relevant and may be employed to navigate the investigated space.

Table 4. Details of ANOVA analysis for polyphenol content in relation to extraction temperature.

	Sum of Squares	df	Mean Square	F-Value	*p*-Value	
Model	4.325×10^5	1	4.325×10^5	174.92	<0.0001	significant
A—Temperature	4.325×10^5	1	4.325×10^5	174.92	<0.0001	
Residual	32,141.30	13	2472.41			
Lack of Fit	29,622.50	9	3291.39	5.23	0.0631	not significant
Pure Error	2518.80	4	629.70			
Cor Total	4.646×10^5	14				
Fit Statistics			R^2	0.9308		
Std. Dev.	49.72		Adjusted R^2	0.9255		
Mean	390.33		Predicted R^2	0.9046		
C.V. %	12.74		Adeq. Precision	30.2539		

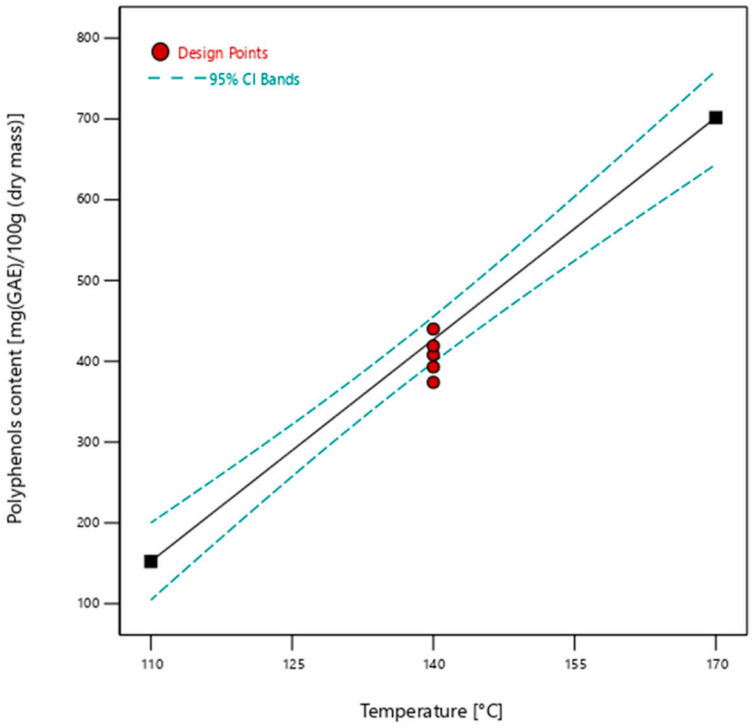

Figure 2. Total polyphenols in relation to extraction temperature.

The total polyphenol content can be calculated using Equation (4).

$$TPC = 9.15504\ T - 854.75194 \quad (4)$$

where TPC—total polyphenol content (mg(GAE)/100 g (dry mass)); T—temperature (°C).

Studies on the amount of polyphenols in subcritical water extracts of the bark of Norway maple (*Acer platanoides* L.) are not currently available. The available information for sugar maple indicates that polyphenol content in leaves varies with harvesting time. A minimum phenolic amount of 105.67 ± 13.16 mg GAE/g dry mass (DM) was obtained by extraction from fall leaves [9]. Other studies indicated that polyphenol content in sugar maple ranged from 35.77 to 136.55 mg GAE/g DM in extracts obtained using microwave-assisted extraction [10] and 292.67 mg GAE/g DM applying acetone as a solvent [14]. The research conducted indicates that polyphenol content extracted from the branch bark of Norway maple ranged from 106 to 943 mg (GAE)/100 g (DM). Variations in the amount of polyphenols present can be attributed to various factors, including but not limited to distinct extraction techniques, process variables, solvent type, duration of material collection for study, and pre-treatment techniques for raw materials [34]. Changes in the environment, including soil type, climate, and geographic location, affect the chemical

structure of phytonutrients [35]. It should be noted that the goal of this study was not to determine the maximum yield for polyphenol extraction, but rather to look into how reactor shape, temperature, and process duration affected the total amount of polyphenols, flavonoids, and antioxidant activity of the extracts that were obtained. A rise in temperature from 110 °C to 170 °C caused a 8.9-fold increase in the amount of polyphenols extracted. The influence of changes in the process duration and the shape of the pressure cell was not observed.

3.3. Total Flavonoid Content (TFC)

Depending on the experimental conditions, the total flavonoid content extracted from the branch bark of Norway maple (*Acer platanoides* L.) ranged from 26 to 188 mg (CTE)/100 g (DM). Figure 3 shows the content of flavonoids in relation to extraction temperature.

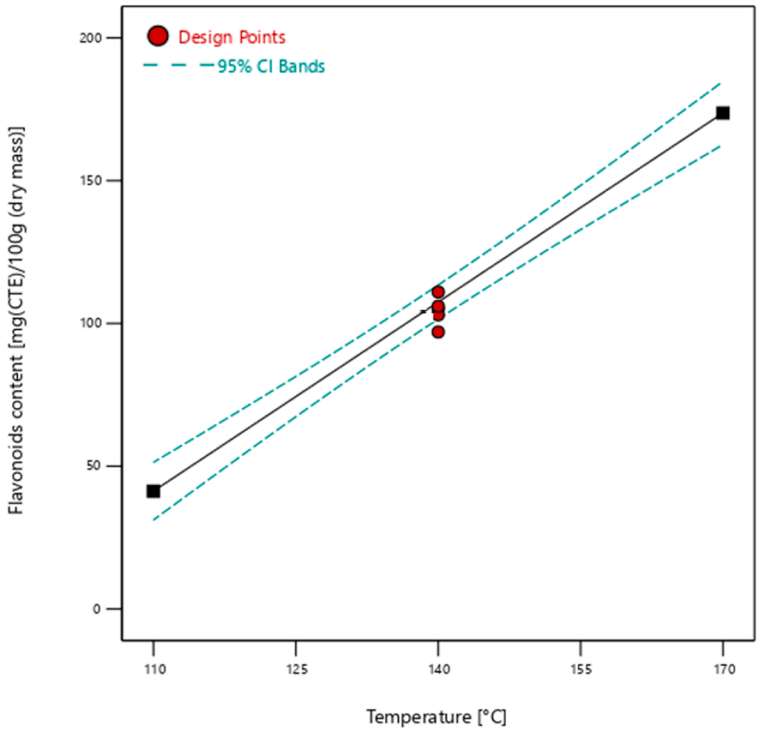

Figure 3. Total flavonoids in relation to extraction temperature.

Based on the analytical results of the flavonoid content, a multivariable analysis of variance was conducted, and the results indicate that the total flavonoids were dependent only on the process temperature. The linear model represents the relation between process temperature and flavonoid content. An increase in the temperature of the process causes an increase in the content of flavonoids in the extract throughout the entire range of the tested parameter. The influence of the reactor shape and process time changes was not observed. Table 5 presents details of the ANOVA analysis.

The model is significant, as indicated by the F-value of 267.82. The likelihood of noise producing an F-value this high is merely 0.01%. *p*-values below 0.05 imply that the model terms are significant. The letter A is a significant model term in this particular case. Considering the pure error, the lack of fit appears not to be significant, as indicated by the 5.91 lack-of-fit F-value. There is a reasonable agreement between the adjusted R^2 (0.9501) and the predicted R^2 (0.9361). Adeq. precision, defined as signal-to-noise ratio, with a value of 34.041, demonstrates an appropriate signal. The resulting model is statistically relevant and may be employed to navigate the investigated space.

Table 5. Details of ANOVA analysis for flavonoid content in relation to extraction temperature.

	Sum of Squares	df	Mean Square	F-Value	*p*-Value	
Model	30,395.51	1	30,395.51	267.82	<0.0001	significant
A—Temperature	30,395.51	1	30,395.51	267.82	<0.0001	
Residual	1475.42	13	113.49			
Lack of Fit	1372.22	9	152.47	5.91	0.0513	not significant
Pure Error	103.20	4	25.80			
Cor Total	31,870.93	14				
Fit Statistics			R^2	0.9537		
Std. Dev.	10.65		Adjusted R^2	0.9501		
Mean	103.07		Predicted R^2	0.9361		
C.V. %	10.34		Adeq. Precision	34.0414		

The total flavonoid content can be calculated using Equation (5).

$$TFC = 2.20705\ T - 201.50641 \quad (5)$$

where TFC—total flavonoid content (mg(CTE)/100 g (dry mass)); T—temperature (°C).

Studies on the amount of flavonoids in subcritical water extracts of the bark of Norway maple (*Acer platanoides* L.) are not currently available. The available information for sugar maple indicates that flavonoid content in leaves varies with harvesting time; a minimum amount of flavonoids of 3.27 ± 0.26 mg CTE/g (DM) was obtained by extraction from fall leaves [9]. Other studies indicated that flavonoid content in sugar maple ranged from 10.51 to 47.33 mg CTE/g DM in extracts obtained using microwave-assisted extraction [10]. The research conducted indicates that flavonoid content extracted from the branch bark of Norway maple ranged from 26 to 188 mg (CTE)/100 g (DM). Variations in the amount of flavonoids present can be attributed to various factors, including but not limited to distinct extraction techniques, process variables, solvent type, duration of material collection for study, and pre-treatment techniques for raw materials [34]. Changes in the environment, including soil type, climate, and geographic location, affect the chemical structure of phytonutrients [35]. It should be noted that the goal of this study was not to determine the maximum yield for flavonoid extraction but rather to look into how reactor shape, temperature, and process duration affected the total amount of polyphenols, flavonoids, and antioxidant activity of the extracts that were obtained. A rise in temperature from 110 °C to 170 °C caused a 7.2-fold increase in the amount of flavonoids extracted, similarly to polyphenols. The influence of changes in the process duration and the shape of the pressure cell was not observed.

3.4. Antioxidant Activity

Depending on the experimental conditions, the antioxidant activity of the branch bark extracts obtained from Norway maple (*Acer platanoides* L.) ranged between 1.29 and 16.24 10^{-6} MTE/1 g (DM). Figure 4 shows the antioxidant activity in relation to process temperature.

Based on the analytical results of the antioxidant activity, a multivariable analysis of variance was conducted, and the results indicate that antioxidant activity is dependent only on the process temperature. The second-order equation represents the relation between process temperature and antioxidant activity. An increase in the temperature of the process causes an increase in antioxidant activity throughout the entire range of the tested parameter. The influence of the reactor shape and process time changes was not observed. Table 6 presents details of the ANOVA analysis.

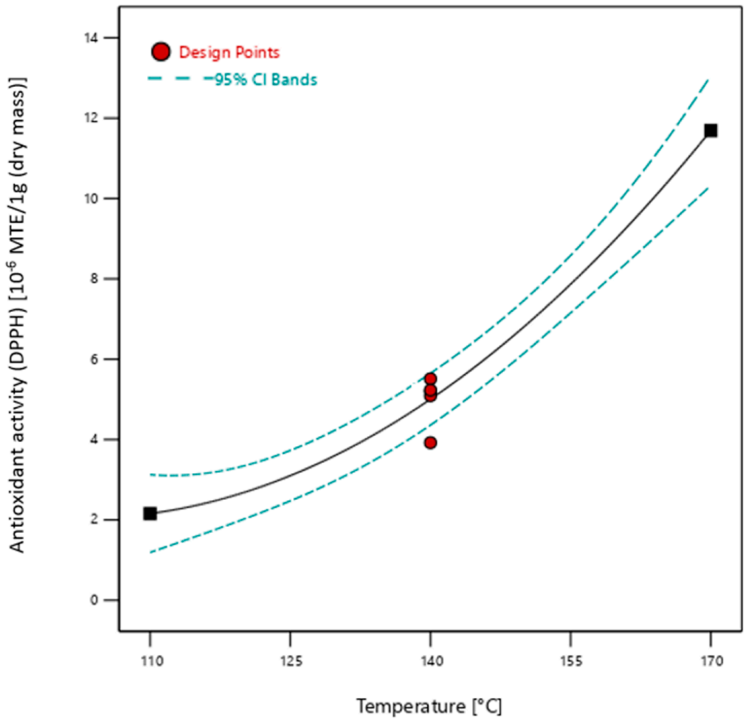

Figure 4. Antioxidant activity in relation to extraction temperature.

Table 6. Details of ANOVA analysis for antioxidant activity in relation to extraction temperature.

	Sum of Squares	df	Mean Square	F-Value	*p*-Value	
Model	121.55	2	60.78	77.20	<0.0001	significant
A—Temperature	51.30	1	51.30	65.16	<0.0001	
A^2	12.35	1	12.35	15.69	0.0019	
Residual	9.45	12	0.7872			
Lack of Fit	7.92	8	0.9900	2.59	0.1866	not significant
Pure Error	1.53	4	0.3818			
Cor Total	131.00	14				
Fit Statistics			R^2	0.9279		
Std. Dev.	0.8873		Adjusted R^2	0.9159		
Mean	5.14		Predicted R^2	0.8749		
C.V. %	17.28		Adeq. Precision	24.0236		

The model is significant, as indicated by the F-value of 77.20. The likelihood of noise producing an F-value this high is merely 0.01%. *p*-values below 0.05 imply that the model terms are significant. The letters A and A^2 are significant model terms in this particular case. Considering the pure error, the lack of fit appears not to be significant, as indicated by the 2.59 lack-of-fit F-value. There is a reasonable agreement between the adjusted R^2 (0.9159) and the predicted R^2 (0.8749). Adeq. precision, defined as signal-to-noise ratio, with value of 24.02, demonstrates an appropriate signal. The resulting model is statistically relevant and may be employed to navigate the investigated space.

The antioxidant activity can be calculated using Equation (6).

$$AC_{DPPH} = 0.002134\ T^2 - 0.438588\ T + 24.58324 \quad (6)$$

where AC_{DPPH}—Trolox equivalent antioxidant activity (10^{-6} MTE/1 g (DM)); T—temperature (°C).

There is currently a lack of information in the literature regarding the antioxidant properties of extracts made from the bark of Norway maples (*Acer platanoides* L.) utilizing a subcritical water extraction method. The available data refers to extracts obtained from

250 g of sugar and red maple bark with a moisture content of 5.6% and 9.5%, as follows: ground to a particle size from 250 to 500 µm; separately processed using 2.5 L of water; processed for a duration of 1 h at 90 °C. In this case, the antioxidant activity (ABTS assay) was 45.20 and 128.71 mmol TE/100 g dry extract [19]. A comparable trend of changes in the reference to the change in process temperature can be seen when comparing the values of the antioxidant activity measured in these studies with the total polyphenol and flavonoid content. In line with the polyphenol and flavonoid content, the antioxidant activity rises as the temperature rises over the whole range of the measured parameter. A rise in temperature from 110 °C to 170 °C caused a 12.6-fold increase in the antioxidant activity, much more than for polyphenol and flavonoid content.

4. Conclusions

Applying the Box–Behnken methodology, the present research examined the effects of the subcritical water extraction parameters of temperature, process duration, and extractor shape on the amount of polyphenols, flavonoids, and antioxidant activity of bark extract from Norway maple (*Acer platanoides* L.). The temperature of the process has a significant impact on the total amount of polyphenols and flavonoids as well as the antioxidant activity of the obtained extracts. The influence of changes in the process duration and the shape of the pressure cell was not observed. A rise in temperature from 110 °C to 170 °C caused a 8.9-fold increase in the polyphenol content, 7.2-fold increase in the flavonoid content, and 12.6-fold increase in the antioxidant activity. The maximum values of polyphenols, flavonoid content, and antioxidant properties were reached at a process temperature of 170 °C, which is the highest point of the temperature variability range observed in these investigations. The study's findings demonstrate how crucial it is to select subcritical water extraction variables carefully in order to achieve the highest extract quality. The temperature rise of the water extraction process in subcritical conditions of Norway maple bark at 170 °C does not result in a decrease in polyphenol and flavonoid content or antioxidant activity. It is suggested that future tests should be performed at higher temperatures, although this may be problematic due to thermal degradation of the raw material. The thermal degradation of extracts was noticeable in previous studies performed on *Juglans regia* L. bark, where a decrease was observed in antioxidant properties, polyphenols, and flavonoid content for temperatures greater than 131.6 °C [36].

Norway maple (*Acer platanoides* L.) bark has not been studied in terms of the influence of process temperature, duration, and reactor shape on the quality of the extract obtained. Chromatographic methods should be used in subsequent research works to examine specific bioactive compounds in order to determine the variability of the chemical composition of the obtained extracts.

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