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Hydrothermal hydrolysis of sweet chestnut (*Castanea sativa*) tannins

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Abstract: Sweet chestnut tannins were treated with subcritical water at temperatures from 120 to 300 °C for reaction times of 15, 30 and 60 min. A great influence of temperature and reaction time on the product yield was noticed. Spectrophotometric methods were used to determine the total tannins, phenols and carbohydrates contents and antioxidant activity. Furthermore, vescalin, castalin, vescalagin, castalagin, 1-*O*-galloyl castalagin, gallic, ellagic and ferulic acids were analysed by HPLC. The results obtained from hydrothermal hydrolysis were compared to results from acid hydrolysis. Finally, the reaction parameters of the hydrothermal hydrolysis process were optimized aimed at obtaining a product with a high concentration of ellagic acid. The optimal conditions for obtaining the highest concentration of ellagic acid of 29.55 % were 250 °C and 5 min. The concentration of ellagic acid in tannin extract obtained by acid hydrolysis was 8.19 %.

Keywords: sweet chestnut; subcritical water; ellagitannins; ellagic acid; gallic acid.

INTRODUCTION

In recent times, all areas of chemistry and chemical engineering are searching for alternatives to reduce negative impacts on the environment. Industrial waste represents a great problem, which besides having a negative influence on the environment, also represents additional costs for industry for their disposal.[1,2] Therefore, the perfect solution is to use waste as a new raw material for the production of various products. Subcritical water technology represents a green alternative technology suitable for the treatment of biomass waste and thus, contributes greatly to the maintenance of a clean and healthy environment.[3]

Water represents universal, non-toxic, cheap and environmentally friendly solvent. At temperatures above 100 °C and below 374 °C, water is still liquid

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under sufficient pressure and it is known as subcritical water.[3] The water at temperatures above 374 °C represents supercritical water.[3] On increasing the temperature and pressure, the properties of water are changed. The increase of temperature has the effect of the breaking of water hydrogen bonds. The water becomes less polar, the dielectric constant of water is lower and thus more similar to organic solvents.[4–6] Another important property of subcritical water is ionic constant, which increases with increasing the temperature to 300 °C and then decreases again.[6,7] Thus, subcritical water can be an important player in acid- and base-catalysed reactions. Furthermore, under subcritical conditions, water molecules have higher kinetic energy and mobility. The consequence is lower density and higher diffusion coefficient.[8] These properties make water a great extraction medium that could replace organic solvents, which may be problematic, especially considering pollution, recycling and costs.[9]

Sweet chestnut (*Castanea sativa*) represents a tree from the *Fagaceae* family.[10] A great amount of sweet chestnut tannins are produced in France, Italy and Slovenia. They have found great uses in different fields: in food, wood and cosmetic industry, in culinary, in medicine, as a fuel, as an addition to animal fodder, used for tanning leather.[9,11] Chestnut wood and bark extracts contain tannins in high amount. Tannins are divided into two groups: condensed and hydrolysable tannins.[11] Condensed tannins, also known as proanthocyanidins, are polymers of flavan-3-ol without sugar residues.[12] There are different sources of proanthocyanidins, such as quebarcho wood, mimosa bark, grape seeds, pine barks and spruce barks. The condensed tannins are important components of food, especially of red wine, green tea and chocolate.[13] Sweet chestnut wood and bark contain a high amount of hydrolysable tannins. Hydrolysable tannins can be classified into two categories: gallotannins and ellagitannins.[11] The gallotannins represent a sugar core substituted with galloyl groups, while ellagitannins are esters of hexahydroxydiphenol groups with a sugar core that often contain galloyl groups.[14,15] The high amount of ellagitannins makes sweet chestnut a very durable tree because they are toxic to microorganisms and can prevent the rapid decay of the wood.[16] It was proved that the structure of chestnut is the most similar to oak.[17] Therefore, chestnut wood is the only alternative to oak that has been approved by the International Organization of Vine and Wine (OIV) for wine ageing.[18] Moreover, chestnut wood was widely used as enological agent, not only because it contains a high amount of ellagitannins and gallic acid, but also due to its widespread availability and lower cost.[18] Vázquez *et al.* have analysed the antioxidant activity and phenolic content of chestnut shell and eucalyptus bark extracts.[9] They showed the composition of these materials and concluded that chestnut shell contains much more extractable compounds and lignin but much less carbohydrates and ash than eucalyptus bark. They also proved that the

antioxidant activity and total phenol content of the extracts were higher in chestnut shell than in extracts of eucalyptus bark.

The aim of this work was the hydrothermal treatment of chestnut tannins in the temperature range 120–300 °C and reaction times of 15, 30 and 60 min. Hydrolysis of sweet chestnut tannins leads to the formation of two important phenolic acids: gallic and ellagic acid. Gallic acid represents an antioxidant with promising therapeutic and industrial applications.[19] Ellagic acid has been of commercial interest in recent years due to many pharmaceutical and industrial application as well as benefits for human health.[20] Furthermore, sugars and their derivatives are also the products of hydrothermal hydrolysis of chestnut tannins, which have high potentials in fuel and polymer applications and applications in many industries (pharmaceutical, agrochemical, flavour, fragrance and food).[21] There are a few articles that are based on the study of the composition of chestnut tannins[10,22–24] and their hydrolysis,[17] but there is no comparable study dealing with subcritical water treatment of chestnut tannins. Furthermore, acid hydrolysis was also made in order to compare these two methods. Therefore, the samples obtained after treatment with subcritical water and acid hydrolysis were analysed by HPLC to determine the degradation products of chestnut tannins. Moreover, the total tannin, phenol and carbohydrate contents and antioxidant activity were evaluated by simple colorimetric methods using spectrophotometer. The reaction mechanism of degradation of sweet chestnut tannin extract by subcritical water was proposed based on the obtained results. The last step of the work was the optimization of the reaction parameters of the process in order to obtain a product with a high content of ellagic acid.

EXPERIMENTAL

Materials

Gallic, ellagic and ferulic acid, Na_2CO_3 and phenol were obtained from Sigma–Aldrich (Germany). Folin–Dennis and Folin–Ciocalteu reagents and sulphuric acid (95–97 %) were purchased from Merck (Germany). Natural sweetened chestnut extract (KPS), which was the material used in this research, was obtained from a local company Tannin Sevnica (Slovenia), as part of neutralized sulphated pyrogallol tannins. The extract was produced from sweet chestnut wood by water extraction. Besides tannins, it contained sodium sulphite, sodium hydroxide and sugars. All other chemicals used for HPLC were of analytical grade.

Acid hydrolysis

A solution was prepared by dissolving 50 mg of the tannin extract in 20 mL of distilled water of which 16 mL were mixed with 5 mL of 6 M HCl and 24 mL of pure ethanol.[25] Acid hydrolysis lasted for 3 h at 90 °C. After 3 h, the reaction mixture was cooled, supplemented with 4 mL of water and analysed by HPLC.

Hydrothermal hydrolysis

The hydrothermal reactions of chestnut tannins were performed in a batch reactor (series 4740 stainless steel, Parr instruments, Moline, IL, USA) at temperatures from 120 to 300 °C and reaction times of 15, 30 and 60 min. Chestnut tannin extract (2 g) was dissolved in 20 mL

of deionised water and poured into the reactor. Nitrogen was used for venting the autoclave and to remove atmospheric oxygen to avoid unwanted side-oxidation reactions. The initial pressure in the autoclave, which was adjusted by nitrogen at room temperature, was 20 bar. As the temperature increases the pressure also increases, thus at 300 °C, it reached the value of 90 bar. In all experiments, the water was in the liquid state. The reactor was heated by an electrical wire. The reaction time was measured from when the temperature was at the desired value. Stirring was applied at 600 rpm. After hydrothermal treatment, the autoclave was rapidly cooled to room temperature and the obtained suspension was filtrated. The obtained water solution was evaporated to dryness under reduced pressure at 40 °C. The water-soluble phase and solid residue were collected. The water-soluble phase was analyzed by HPLC and spectrophotometric methods. The yield of products was calculated by Eq. (1) and expressed in %:

$$Y_i / \% = 100 \frac{m_i}{m_0} \quad (1)$$

where m_i represents mass of water-soluble phase (m_{WS}) or mass of solid residue (m_{SR}), while m_0 represents the mass of the initial material.

Methods for determination of total tannins, total phenols, total carbohydrates and antioxidant activity, as well as HPLC method for analysis of water-soluble samples are described in the Supplementary material to this paper.

Optimization of the reaction parameters

Response surface methodology, *i.e.*, central composite design (CCD), was chosen for the optimization of reaction parameters of hydrothermal process for hydrolysis of chestnut tannin extract. The two variables were optimized: temperature (X_1, °C) in the range from 150 to 250 °C and reaction time (X_2, min) in range from 15 min to 60 min. The desired outcome was to optimize reaction parameters to obtain the highest concentration of ellagic acid.

The whole design consists of 13 experimental points. Five replicates of centre points were used for the determination of the pure error sum of squares.

Using CCD to optimize the process includes: a) proposal of experiments in a certain range of factor variables and their performance; b) suggestion of the mathematical model based on results obtained from the experiments, as well as graphs; c) checking the adequacy of the model; d) determination of the optimal point of the process and comparison with the obtained experimental value.

RESULTS AND DISCUSSION

Effect of temperature and reaction time on the yield of water-soluble and solid products

It has already been mentioned that the temperature and reaction time under subcritical water conditions have significant influences on the product yield. The effect of temperature for reaction times of 15, 30 and 60 min on the yield of water-soluble product is shown in Fig. 1A. The results show that the yield of water-soluble product decreased with increasing the temperature and reaction time. Therefore, the maximal yield of water-soluble product was 96.12 % obtained when the tannin extract was treated with subcritical water at a temperature of 120 °C and a reaction time of 15 min.

The yield of solid residue in dependence on different temperatures for reaction times of 15, 30 and 60 min is shown in Fig. 1B. The yield of solid residue increased with temperature as well as with reaction time. The exception was the temperature of 300 °C, where the yield of solid residue decreased after 15 min. At temperatures from 150 to 180 °C, the solid residue drastically increased and at a temperature of 300 °C, reached the maximal value of 39.62 % in 15 min, after which it decreased with increasing reaction time.

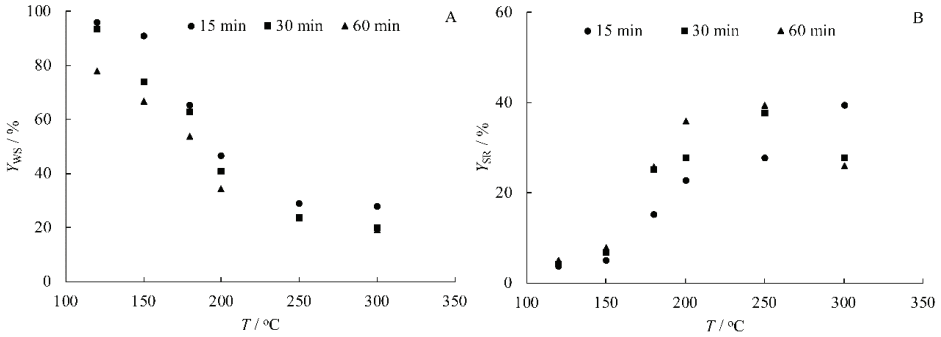

Fig. 1. The effect of temperature on: A – the yield of the water-soluble fraction and B – the yield of solid residue, for reaction times of 15, 30 and 60 min.

It is evident that the water-soluble compounds were the major products of hydrothermal treatment of sweet chestnut tannin at temperatures up to 150 °C, where the amount of char was at the trace level. These results suggest that conversion of chestnut tannin at low temperatures occurred only through reactions of dissolution and hydrolysis to water-soluble carbohydrates and further to organic acids. Secondary reactions of the water-soluble fraction to char and gases were obvious at temperatures higher than 150 °C. As the yield of water-soluble product started to decrease as the temperature increased, char formation by condensation and re-polymerization of these liquid products was favoured.

Composition of the water-soluble product

Total tannins, phenols and carbohydrates content and antioxidant activity. The total content of tannins in the water-soluble product in dependence of temperature for reaction times of 15, 30 and 60 min is presented in Fig. 2A. The total tannins content increased with increasing temperature and time at up to 200 °C. At 250 °C, it increased from 15 to 30 min and reached the maximal value of 70.19 %, after which it decreased. At 300 °C, the total tannins content decreased for all reaction times. The initial material contained 43.70 % of total tannins. It could be concluded that tannin extract was degraded under hydrothermal conditions into new simpler tannin compounds. The total tannins content in the product obtained by acid hydrolysis was quite low and amounted to 1.79 %.

Similar results as in the case of total tannins were obtained for the total content of phenols in the water-soluble product, as shown in Fig. 2B. The initial material contained 45.20 % of total phenols. As in the case of the total tannins, the content of total phenols increased with temperature and reaction time up to 200 °C. At 250 °C, it increased from 15 to 30 min, where the maximum of 72.93 % was reached, and then it decreased. At 300 °C, it decreased with increasing reaction time. However, the total phenols content in the product obtained by acid hydrolysis was extremely high and amounted to 87.4 %. It could be supposed that on acid hydrolysis, almost all ellagitannins were hydrolysed mainly into simple phenolic compounds, while by hydrothermal hydrolysis, the phenolic compounds were probably further degraded to other organic compounds (aldehydes, ketones, alkanes, alkenes, *etc.*).

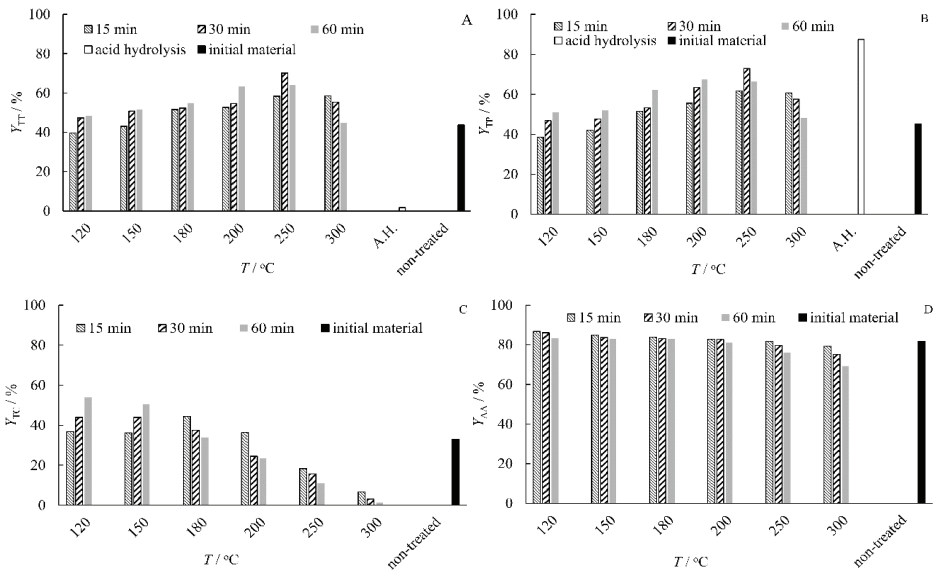

Fig. 2. A – The content of total tannins; B – the content of total phenols; C – the content of total carbohydrates; D – the antioxidant activity of the water-soluble product in dependence on temperature for reaction times of 15, 30 and 60 min.

The content of total carbohydrates in water-soluble product in dependence of temperature for different reaction time is presented in Fig. 2C. The total carbohydrates content in the initial material was 32.88 %. The content of total carbohydrates increased with reaction time at 120 and 150 °C. The maximal value of total carbohydrates (53.95 %) was obtained at 120 °C after 60 min. When the temperature exceeded 180 °C, the carbohydrates content decreased with reaction time and temperature. It could be concluded that above 180 °C, the degradation of sugars into furfurals, aldehydes, ketones and organic acids occurred.

The antioxidant activity of water-soluble product in dependence on temperature for different reaction time is presented in Fig. 2D. The antioxidant activity of initial material was 81.72 %. After hydrothermal treatment of the material at 120–200 °C for 15 min, the antioxidant activity slightly increased. However, with further increasing the reaction time, it started to decrease. The maximal value of the antioxidant activity (86.75 %) was obtained at 120 °C after 15 min, while the minimal value was 69.2 %, obtained at 300 °C after 60 min.

HPLC analysis of tannins and derivatives. The effects of temperature and reaction time on the concentration of ellagitannins are presented in Table I, while Table II presents the concentrations of gallic and ellagic acid in dependence on the reaction parameters. The most abundant compounds in the initial material were castalagin (4.18 mass %) and vescalagin (5.65 mass %), followed by 1-*O*--galloyl castalagin (2.48 mass %) and gallic acid (3.33 mass %). The contents of vescalin (0.848 mass %), castalin (0.33 mass %) and ellagic acid (0.57 mass %) were lower. As could be observed, the concentrations of gallic acid and 1-*O*-galloyl castalagin slightly increased with increasing time only in the case when the tannin extract was treated with water at a temperature of 120 °C. On the other hand, ellagic acid was present in trace amounts under these conditions. However, as the temperature and reaction time increased, the concentrations of gallic acid and ellagitannins decreased, while the content of ellagic acid increased. Gallic acid was not present in the products obtained above 200 °C, while ellagitannins were not detected in products obtained at temperatures higher than 150 °C. The highest amount of ellagic acid (24.72 mass %) was obtained at a temperature of 250 °C and reaction time of 15 min. With further increasing of the temperature and reaction time, the concentration of ellagic acid dramatically decreased. At a temperature of 300 °C and a reaction time of 60 min, no ellagic acid was detected in the product. Ferulic acid started to appear in a very low amount at a temperature of 300 °C. It could be assumed that its concentration would increase with further increasing of the reaction time or temperature.

TABLE I. The effect of temperature and reaction time on the concentration of ellagitannins (mass %)

Compound	Conditions						Acid hydrolysis	Non-treated
	120 °C			150 °C				
	Time, min							
	15	30	60	15	30	60		
Vescalin	0.64	0.44	0.40	0.35	0.32	0.30	–	0.85
Castalin	0.42	0.38	0.35	0.36	0.25	0.18	–	0.33
Vescalagin	5.85	5.35	4.92	3.31	3.01	1.03	0.75	5.65
Castalagin	3.77	3.58	3.42	2.21	1.94	0.93	0.37	4.18
1-*O*-galloyl castalagin	2.63	2.73	2.87	3.00	2.89	1.12	0.29	2.48

TABLE II. The effect of temperature and reaction time on the concentration of gallic and ellagic acid (mass %)

Temperature, °C	Time, min	Component	
		Gallic acid	Ellagic acid
120	15	3.24	0.69
	30	3.45	0.73
	60	3.67	0.83
150	15	2.73	1.29
	30	2.46	1.35
	60	2.18	3.58
180	15	2.55	6.24
	30	1.75	7.80
	60	1.32	8.27
200	15	0.32	9.04
	30	0.18	12.35
	60	/	20.13
250	15	/	24.72
	30	/	21.48
	60	/	6.99
300	15	/	8.54
	30	/	2.07
	60	/	/
Acid hydrolysis		0.86	8.19
Non-treated		3.33	0.57

The acid hydrolysis of chestnut tannins mostly led to formation of ellagic acid with a concentration of 8.19 mass %, while gallic acid occurred in a small amount (0.86 mass %). Concentrations of vescalagin, castalagin and 1-*O*-galloyl castalagin in acid hydrolysed product were 0.75, 0.37 and 0.29 mass %, respectively, while vescalin and castalin were not present.

The proposed mechanism of degradation of sweet chestnut tannin extract by subcritical water and the formation of important products is described in Fig. 3.[17,26–28] Ellagitannins are biologically formed from pentagalloyl-glucose (gallotannin).[26] Canas *et al.* assumed that the gallic acid is a product of the hydrolysis of some galloyl esters associated with the parietal composites of chestnut cells.[29]

Vescalagin and castalagin are two aromatic glycosides with a 4,6-hexahydroxydiphenyl coupling and an unique flavogallonyl group, which is composed of three galloyl groups linked together with C–C bonds.[17] The presence of vescalin and castalin is caused by hydrolysis of the hexahydroxydiphenyl unit of vescalagin and castalagin.[17] Ellagitannins are converted into ellagic acid by an intra-molecular esterification reaction of hexahydroxydiphenic acid.[30] 1-*O*-galloyl castalagin is hydrolysable tannin previously identified in *Eugenia grandis*.[31] Comandini *et al.* deducted that this molecule could be formed by esterification of castalagin or vescalagin with a gallic acid residue.[22]

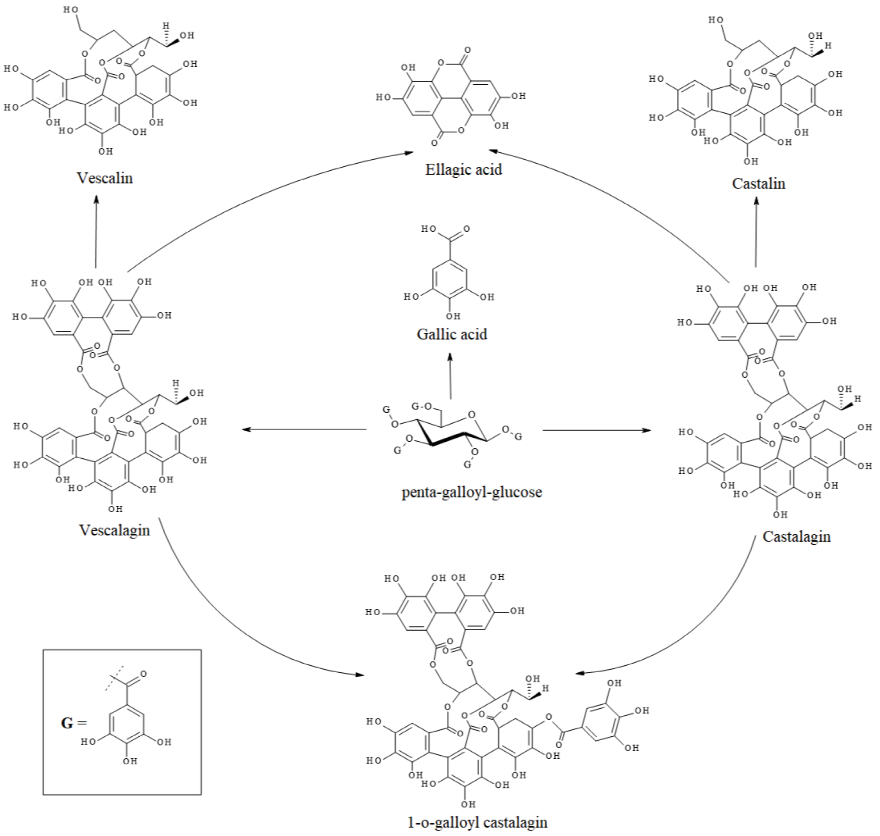

Fig. 3. The proposed mechanism of the reaction of sweet chestnut tannins in subcritical water.

It was proved that vescalagin and castalagin can react with themselves to produce oligomeric ellagitannins, such as roburin A and roburin D (dimers of vescalagin and castalagin, respectively).[32]

Optimization of the reaction parameters

To obtain the highest concentration of ellagic acid, the hydrothermal hydrolysis process of the tannin extract was optimized using the response surface methodology, exactly by central composite design (CCD). The proposed experiments depending on two factors temperature (X_1, °C) and reaction time (X_2, min), as well as experimental and predicted concentrations of ellagic acid, are presented in Table III.

A quadratic model with natural logarithmic-transformed response to obtain concentration of ellagic acid (Y_{EA}) was suggested as the best-fitted model:

$$\ln Y_{EA} = 2.44 + 0.84X_1 + 0.071X_2 - 0.59X_1X_2 - 0.77X_1^2 + 0.068X_2^2 \quad (1)$$

where X_1 and X_2 represent coded factors of the temperature and reaction time, respectively.

TABLE III. CCD matrix and the experimental and predicted response values for the concentration of ellagic acid

Std. order	Independent variable		Y_{EA}: Concentration of ellagic acid, mass %	
	X_1: temperature, °C	X_2: time, min	Experimental value	Predicted value
1	–1 (150.00)	–1 (15.00)	1.29	1.27
2	+1 (250.00)	–1 (15.00)	24.72	22.13
3	–1 (150.00)	+1 (60.00)	4.25	4.75
4	+1 (250.00)	+1 (60.00)	7.84	7.84
5	–1.41 (129.29)	0 (37.50)	0.785	0.760
6	+1.41 (270.71)	0 (37.50)	7.50	8.11
7	0 (200.00)	–1.41 (5.68)	10.80	11.88
8	0 (200.00)	+1.41 (69.32)	15.61	14.52
9	0 (200.00)	0 (37.50)	12.17	11.47
10	0 (200.00)	0 (37.50)	11.67	11.47
11	0 (200.00)	0 (37.50)	10.42	11.47
12	0 (200.00)	0 (37.50)	11.87	11.47
13	0 (200.00)	0 (37.50)	11.13	11.47

The regression coefficients of the intercept, linear, quadratic and interaction terms of the model were significant model terms and are presented in Table IV. Table IV also shows the analysis of variance (ANOVA) of the experimental results of the CCD. The high *F*-value of the proposed model (260.60) implied that the model is significant. *F*-value of 4.11 and *p*-value of 0.1027 of lack of fit showed insignificant lack of fit relative to the pure error. The value R^2 was 0.9947, which confirmed good correlation between the experimental and pre-

TABLE IV. Estimated regression coefficients for the determined model and the analysis of the variance (ANOVA) of the experimental results

Parameter	Coefficient estimate	Standard error	Sum of squares	Degrees of freedom	Mean square	*F*-value	*p*-value probability>*F*
Model			11.45	5	2.29	260.60	<0.0001
Intercept	2.44	0.042					
X_1	0.84	0.033	5.71	1	5.71	649.42	<0.0001
X_2	0.071	0.033	0.040	1	0.040	4.54	0.0706
$X_1 X_2$	–0.59	0.047	1.37	1	1.37	155.85	<0.0001
X_1^2	–0.77	0.036	4.13	1	4.13	470.38	<0.0001
X_2^2	0.068	0.036	0.032	1	0.032	3.64	0.0982
Residual			0.062	7	0.008788		
Lack of fit			0.046	3	0.015	4.11	0.1027
Pure error			0.015	4	0.003765		
R^2	0.9947		Adj. R^2	0.9908			
C.V. %	4.68		Pred. R^2	0.9693			
PRESS	0.35		Adeq. prec.	53.265			

dicted values of the ellagic acid concentrations and also the quality of the model. The predicted R^2 value of 0.9693 was in reasonable agreement with the adjusted R^2 value of 0.9908. Furthermore, the coefficient of variation ($C.V.$ = 4.68 %) was reproducible, due to its value being below 5 %. The value of adequate precision of 53.265, which measures the signal to noise ratio, was adequate, due to its value being greater than 4. This analysis of the model showed that the experimental results fit to the proposed model, which could be used to navigate the design space.

The graphical representations of the model to calculate the concentration of ellagic acid as: A) the three-dimensional response surface and B) the two-dimensional contour plot are shown in Fig 4.

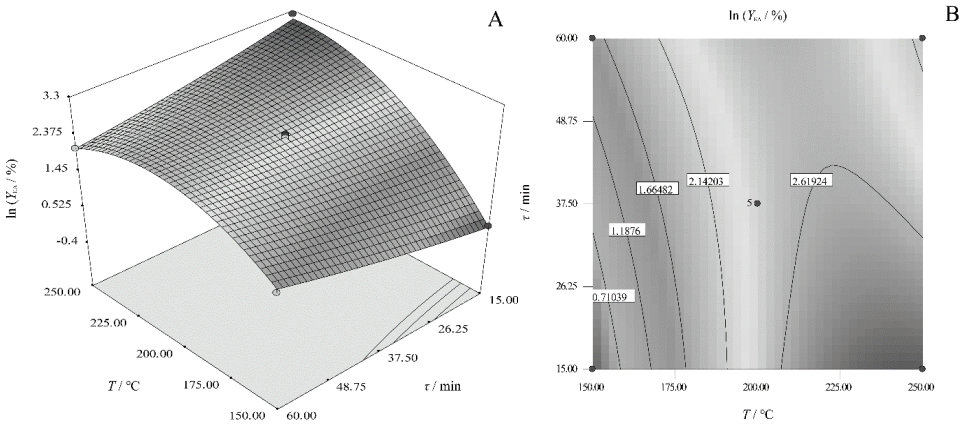

Fig. 4. Graphical representations of the model to calculate concentration of ellagic acid as: A – the three-dimensional response surface and B – the two-dimensional contour plot.

The maximal concentration of ellagic acid predicted by the model was 22.13 mass %, obtained at 250 °C and 15 min. Due to the fact that at 250 °C the concentration of ellagic acid increased almost linearly with decreasing reaction time, the obtained mathematical model was extrapolated to lower reaction times and the calculated optimum values of the variables were a temperature of 250 °C and a reaction time of 5 min, when the concentration of ellagic acid was 29.82 mass %. Experiments were performed under determined optimized conditions, to be compared with predicted results. The obtained experimental value of ellagic acid concentration was 29.55 mass %, which showed the validity of the suggested model due to insignificant difference between the experimental and predicted values.

CONCLUSIONS

The aim of the present work was to study the hydrothermal hydrolysis of sweet chestnut tannins and to optimize this process in order to obtain a product

with high concentration of ellagic acid. Thus, the effect of temperature from 120 to 300 °C and reaction times of 15, 30 and 60 min on the hydrothermal degradation of chestnut tannins was observed. The impact of these parameters on product yield was obvious. The maximal amount of water-soluble fraction was 96.12 % obtained at temperature of 120 °C and 15 min. The highest yield of solid residue was 39.62 % at 300 °C and 15 min. The yield of the water-soluble product decreased with increasing temperature and reaction time, while the amount of solid residue generally increased as the temperature and reaction time increased. The spectrophotometric methods used for analysis of the water-soluble product obtained by hydrothermal hydrolysis showed an increase in the contents of total tannins and phenols up to 250 °C and 30 min and thereafter the content of total tannins and phenols decreased with further increasing of the temperature and reaction time. The content of carbohydrates increased with increasing reaction time from 120 to 150 °C and then decreased with further increasing of the temperature and reaction time. The antioxidant activity decreased with increasing reaction time and temperature. Therefore, the maximal antioxidant activity of 86.75 %, which was slightly higher than that of the starting material, was obtained for the water-soluble product produced at 120 °C and 15 min.

Based on a HPLC method found in the literature,[22] the determined compounds were vescalin, castalin, vescalagin, castalagin, 1-*O*-galloyl castalagin, gallic, ellagic and ferulic acid. The content of ellagitannins (castalagin, vescalagin, 1-*O*-galloyl castalagin, castalin and vescalin) in the water-soluble product obtained by hydrothermal hydrolysis, as well as the gallic acid content, decreased with increasing temperature and reaction time. On the contrary, the ellagic acid content reached the maximal value of 24.72 mass % at a temperature of 250 °C and reaction time of 15 min when the tannin extract was treated by subcritical water. Furthermore, the conventional acid hydrolysis of chestnut tannin extract was also performed for comparison and the results showed that the tannins content decreased to 1.79 %, while the phenols content increased to 87.4 %. The concentration of ellagic acid in the sample obtained by acid hydrolysis was 8.19 mass %, while concentrations of gallic acid and ellagitannins were quite low. Vescalin and castalin were not present in samples obtained by acid hydrolysis. Ferulic acid occurred in a trace amount in the water-soluble products obtained by hydrothermal hydrolysis at higher temperatures, and it could be assumed that its content would increase with further increases in the temperature and reaction time.

By using CCD experimental design and fitting experimental data in the temperature range from 150 to 250 °C and reaction time from 15 min to 60 min, a quadratic mathematical model for calculating the concentration of ellagic acid was determined. By extrapolation of the mathematical model to lower reaction times, the optimal reaction conditions for obtaining the highest concentration of ellagic acid were calculated as 250 °C for 5 min giving a concentration of ellagic

acid of 29.82 mass %. The experimentally determined concentration under these conditions was of 29.55 mass %, which confirmed the validity of the suggested model because the difference between experimental and predicted values was not significant.

It could be concluded that these results could serve as guidelines and the basis for further research. In the present work, it was shown that by hydrothermal hydrolysis of sweet chestnut tannins it is possible to obtain a product with a high concentration of ellagic acid and no gallic acid. Based on these results, it could be concluded that the reaction conditions favour the formation of ellagic acid that is much more stable under these conditions than gallic acid. Nevertheless, further research on tannin hydrolysis with subcritical water will be required in order to investigate the reaction mechanism and the kinetics of the reaction.

SUPPLEMENTARY MATERIAL

Additional data is available electronically at the pages of the website of the journal: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

ХИДРОТЕРМАЛНА ХИДРОЛИЗА ТАНИНА СЛАТКОГ КЕСТЕНА (*Castanea sativa*)

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Танини слатког кестена (*Castanea sativa*) су третирани субкритичном водом на температурама од 120 до 300 °C и реакционим временима од 15, 30 и 60 min. Састав добијених производа је анализиран применом HPLC. Значајан утицај температуре и реакционог времена на принос производа је примећен. Спектрофотометријске методе су коришћене за одређивање тоталних танина, фенола, угљених хидрата и антиоксидативне активности. Осим тога, анализирана једињења применом HPLC су: вескалин, касталин, вескалагин, касталагин, 1-*O*-галоил касталагин, гална, елагна и ферулична киселина. Резултати добијени из хидротермалне хидролизе су упоређени са резултатима из киселинске хидролизе. Коначно, оптимизација реакционих параметара хидротермалног процеса хидролизе је начињена с циљем да се добије производ са високом концентрацијом елагне киселине. Оптимални услови за добијање највише концентрације елагне киселине од 29,55 % су 250 °C и 5 min. Концентрација елагне киселине у екстракту танина добијена киселинском хидролизом је била 8,19 %. Осим тога, киселинска хидролиза је дала драстично нижи садржај тоталних танина у поређењу са хидротермалном хидролизом танина, због деградације танина у једноставнија фенолна једињења.

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