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Conventional and ultrasound-assisted extraction of (poly)phenols from elderberry flowers *Sambucus Nigra L.*

Nebojša Vasiljević[1] | Vladan Mićić[1] | Milorad Tomić[1] | Dragica Lazić[1] | Ljubica Vasiljević[1] | Duško Kostić[1]

[1]University of East Sarajevo, Faculty of Technology Zvornik, Karakaj 34a, 75400 Zvornik, Republic of Srpska, Bosnia and Herzegovina

Correspondence
Nebojša Vasiljević
Email: nebojsavasiljevic96@gmail.com

Abstract

In this paper, conventional solid-liquid extraction and ultrasound-assisted extraction were utilized for (poly)phenols extraction from black elderberry flowers. The extraction lasted 30 to 120 min, with a solid-to-solvent ratio of 1:15 w/v and 1:30 w/v and solvents of 30% and 60% ethanol. Total (poly)phenols, flavonoids and anthocyanins contents were measured using the Folin-Ciocalteu reagent, aluminium chloride and the pH differential methods, respectively. The results show that the extraction process has the highest velocity in the first 30 min, when the most (poly)phenols are extracted, but it becomes slower as time passes. Higher yields are obtained by utilizing a solid-to-solvent ratio of 1:30 w/v, which indicates that when the amount of drug increases over a certain optimal value, the resistance to mass transfer from a solid material to liquid increases. Also, higher yields are obtained by using a solvent that contains a higher content of ethanol. Finally, it was discovered that ultrasonic extraction provides to higher (poly)phenolic compound yields and can substitute conventional extraction methods.

Keywords: anthocyanins, elderberry extracts, flavonoids, (poly)phenols

1. INTRODUCTION

European black elderberry (*Sambucus nigra L.*), which belongs to the Adoxaceae family, is an extremely accessible and abundant plant native to the northern hemisphere. Their seeds are spread rapidly by birds and other animals to colonize forest edges and disturbed areas, leading to being nowadays diffused in different habitat as subtropical regions of Asia, North Africa and North America (Domínguez et al. 2020; Finn, Thomas, Byers, & Serçe 2008).

The Food and Drug Administration (FDA) has authorized the flowers of *Sambucus canadensis* and *S. nigra* as generally Recognized as Safe (GRAS) for use as flavoring agents. Elders, particularly *S. nigra*, are native to Europe and they are used as a colorant or flavoring in juices and wines atkinson2002. The flowers and berries of *S. nigra* are regarded medicinal parts, although elderberry flowers have been certified by German Commission E for colds, while berries, leaves, and barks have not been recognized by WHO, ESCOP, or German Commission E (Ulbricht et al. 2014). Elderberry contains bioactive components, particularly (poly)phenols such as flavonols, phenolic acids, proanthocyanins, and anthocyanins, which give the fruit its distinctive black-purple color (Anton et al. 2013). The predominant anthocyanin of *S. nigra* is cyanidin 3-glucoside, depending on the variety and fruition (204.6–481.4 mg CGE/100 g fruits). In turn, the second anthocyanin in terms of the content was cyanidin 3-sambubioside (122.2–269.1 mg CGE/100 g fruits) (Lee & Finn 2007). The predominant flavonols were quercetin, kaempferol and isorhamnetin. Flavonols derived from elderberry occur as glycosides of rutin and glucose; moreover, acylated quercetins were also present (Christensen, Kaack, & Fretté 2008).

(Poly)phenolic compounds are present in the leaves, fruits and flowers. The flowers of elderberry contained tenfold more flavonols (214.25 mg/100 g) than fruits (20.18 mg/100 g) and several times more than the leaves (17.01 mg/100 g) (Dawidowicz, Wianowska, & Baraniak 2006). Elderberry flowers and fruits, apart from flavonols, contain large amounts of pheno-

lic acids. Fruits contain chlorogenic, crypto-chlorogenic and neochlorogenic acids, as well as trace levels of ellagic acid (0.04 mg/100 g) (Fazio, Plastina, Meijerink, Witkamp, & Gabriele 2013). Elder flowers also contain N-phenylpropenoyl-l-amino acid amides, which strongly stimulate mitochondrial activity and cell proliferation in human keratinocytes and liver cells and inhibit *Helicobacter pylori* adhesion to the human stomach without causing necrotic toxicity (Hensel et al. 2007).

Conventional techniques such as heat reflux and Soxhlet extraction can be used to extract (poly)phenolic compounds from various matrices. However, there are disadvantages, including the consumption of large volumes of solvent and energy, lengthy extraction times, and the potentially deleterious degradation of labile compounds (Yang, Wei, Huang, Lee, & Lin 2013). At present, proposed extraction methods capable of overcoming the above-mentioned drawbacks include ultrasound-assisted extraction – UAE (Briars & Paniwnyk 2013) and microwave-assisted extraction (Liao, Wang, Liang, Zhao, & Jiang 2008). Among these methods, UAE has been widely employed in the extraction of target compounds from different materials, owing to its facilitated mass transport of solvent from the continuous phase into plant cells (Vinatoru 2001). In addition to using less solvents, UAE requires shorter lead time, less energy, and can be combined with other extraction methods to enhance extraction efficiency (Da Silva, Nunes, & Hoskin 2023).

The aim of this paper was to investigate at how process parameters (such as type of extraction, extraction time, solid-to-solvent ratio, and ethanol content in the extraction solvent) affected the yield of (poly)phenolic components in an ethanol extract of black elderberry flower.

2. MATERIALS AND METHODS

Crushed black elderberry flower, which was purchased from local health food store, was used for the extraction. For sample extraction, various concentrations of ethanol were used, while extract characterization was performed using the following reagents: Folin-Ciocalteu reagent (Carlo Erba, Germany), sodium carbonate (Lach:ner, Czech Republic), gallic acid (Sigma Aldrich, USA), aluminum chloride (Lach:ner, Czech Republic), sodium hydroxide (Lach:ner, Czech Republic), sodium nitrite (Zorka Šabac, Serbia), catechin hydrate (Sigma Aldrich, USA), and potassium chloride buffer pH=1.0 (Lach-Ner, Czech Republic).

Classic maceration procedure (extraction without mixing) and ultrasound-assisted extraction were used to extract (poly)phenolic compounds from the sample. The process was carried out at room temperature in laboratory beakers where the plant is placed together with the solvent.

The procedure was performed under the following process conditions:

- Extraction time [min] – 30, 45, 60, 90 and 120,
- Solid-to-solvent ratio [w/v] – 1:15 and 1:30 and
- Percentage of ethanol in the extraction solvent [vol. %] – 30 and 60.

The method for total (poly)phenols determination is based on oxidation-reduction reactions involving hydroxyl groups of phenol and the Folin-Ciocalteu reagent, as well as polymer complex ions of molybdenum and tungsten. The reaction requires a basic environment, which is created by adding sodium carbonate to the reaction mixture. The measurement is spectrophotometric, at wavelength of 765 nm, and gallic acid is utilized as the standard ISO (2005). A Shimadzu 1800 spectrophotometer was utilized for spectrophotometric determination, with the calibration curve ranging from 50 to 500 mg/l of gallic acid. The results are given in milligrams of gallic acid equivalent per gram of plant material (mg GAE/g).

The flavonoids content of the sample is determined using the colorimetric technique with aluminum chloride. In an acidic solution, aluminum chloride forms stable complexes with the C-4 keto group or the C-3 and C-5 hydroxyl groups of the present flavones and flavonols, and unstable complexes with orthodihydroxyl groups in the A or B ring of flavonoids. Measurement is spectrophotometric at wavelenght of 510 nm, with catechin hydrate as the standard (Savi, Dos Santos, Gonçalves, Biesek, & De Lima 2017). For determination of flavonoids the calibration curve was in range 20 to 200 mg/l of catechin hydrate. The results are given in milligrams of catechin hydrate equivalents per gram of plant material (mg CTH/g).

The quantitative determination of total anthocyanins (non-degraded monomers and products of their degradation) is based on the property of anthocyanins to reversibly change their structure when the pH of the environment changes, which also changes the absorption spectrum. The content of total anthocyanins is determined by the "single" method, described in the paper (Giusti & Wrolstad 2001), which is based on measuring the absorbance of the anthocyanin solution at pH=1. The total anthocyanin concentration in the sample is determined as cyanidin-3-glucoside equivalent (mg Cy3G/g).

Statistical analysis was performed using MINITAB 21. Given that a high rate of extraction is anticipated at first, followed by a gradual slowing (López, Brousse, & Linares 2023), a concave function was employed to approximate

Table 1. Content of (poly)phenols, flavonoids and anthocyanins in the extracts

Type of mixing	Solid-to-solvent ratio (w/v)	Ethanol (%)	Extracted species								
			(poly)phenols			flavonoids			anthocyanins		
			extraction time (min)								
			30	60	120	30	60	120	30	60	120
UAE	1:15	30	19.37	31.23	36.83	12.49	20.47	23.40	0.060	0.096	0.126
		60	28.48	35.42	43.13	19.58	29.86	35.85	0.479	0.572	0.712
	1:30	30	41.03	47.00	47.12	19.00	23.89	28.38	0.100	0.164	0.228
		60	49.26	52.51	59.18	31.68	35.11	37.78	0.633	0.700	0.972
No mixing	1:15	30	22.52	30.50	33.79	10.52	17.47	18.32	0.050	0.071	0.080
		60	27.28	30.85	39.45	15.68	18.45	20.45	0.433	0.500	0.593
	1:30	30	34.80	41.73	43.67	14.85	16.20	17.47	0.085	0.160	0.185
		60	38.85	40.45	44.18	14.91	16.11	18.64	0.473	0.597	0.657

the experimental data. The formula for that function is:

$$Y = a \cdot X/(b + X)$$

where are:

- Y – (Poly)phenols (flavonoids or anthocyanins) content [mg/g],
- X – Time [min] and
- a, b – coefficients.

By choosing a confidence level of 95%, the coefficients a and b in the previous concave function were determined. The algorithm used is Gauss-Newton, the maximum number of iterations is 200, while the tolerance is 0.00001.

3. RESULTS AND DISCUSION

Table 1 shows the results for the content of (poly)phenols, flavonoids and anthocyanins for three different extraction times.

The statistical program MINITAB 21 was used to determine the equation for the dependence of the (poly)phenols yield in the extract on the extraction time. The results of the statistical analysis are shown in Table 2.

Figure 1 depicts the time dependence of extracted (poly)phenols under constant other process parameters (solid-to-solvent ratio and percentage of ethanol in the extraction solvent). Also, the diagrams show curves for the yield of (poly)phenols using ultrasound-assisted extraction and extraction using the maceration technique (extraction without mixing). Since the parameters solid-to-solvent ratio and percentage of ethanol in the extraction solvent have two levels per experimental design setup, a total of $2^2=4$ combinations will be utilized to illustrate the time dependency of total (poly)phenolic yield.

Comparing the curves for ultrasound-assisted extraction (UAE) and extraction without mixing from Figure 1, it is noticed that UAE produces better (poly)phenols yields. However, there are considerable differences when looking specifically at process conditions. For example, after 120 min of extraction, by comparing the lower values of the solid-to-solvent ratio and the ethanol content in the solvent (Figure 1a) and the upper values of the solid-to-solvent ratio and the ethanol content in the solvent (Figure 1d), and when employing ultrasound-assisted extraction instead of extraction without mixing, the (poly)phenols content is increased by just 8.2% at lower values (33.79 mg GAE/g without mixing and 36.83 mg GAE/g with UAE), but by up to 25.3% at higher levels (44.18 mg GAE/g without mixing and 59.18 mg GAE/g with UAE).

Time has a great influence on the extraction of (poly)phenolic compounds from the sample. There are two different extraction periods: the initial period (in the first 30 minutes of extraction), in which (poly)phenolic compounds are intensively extracted from the sample, and the second, stable period, in which the rate of extraction is much slower. The obtained results are in agreement with the literature data, where the concentration of the extracted biologically active components increased with prolonged exposure time (Jovanović et al. 2017).

However, looking at the extraction periods separately, there is a different effect of the solid-to-solvent ratio and the ethanol content in the solvent on the extraction rate. This is best seen by comparing Figure 1a (lower values of solid-to-solvent ratio and ethanol content in the solvent) and Figure 1d (upper values of solid-to-solvent ratio and ethanol content in the solvent) during ultrasound-assisted extraction. First of all, with a solid-to-solvent ratio of 1:15 w/v and the use of 30% ethanol, the velocity of extraction in the initial period is slower, and after 30 minutes, only 19.37 mg GAE/g of (poly)phenols are extracted. However, in the next 90 minutes, there is a further increase in (poly)phenols in the extract, and its content increases by an additional 47.4% (up to a value of 36.83 mg GAE/g). The opposite effect exists with the use of a solid-to-solvent ratio of 1:30 w/v and

Table 2. Determination of coefficients a and b in equation Y=a·X/(b+X) for (poly)phenols content

Constant values	Conventional extraction (without mixing)				Ultrasound-assisted extraction			
	Summary		Coefficients		Summary		Coefficients	
Solid-to-solvent ratio of 1:15 and 30% ethanol	Iterations Final SSE[a] DFE[b] MSE[c] S[d]	8 4.33 4 1.08 1.04	a b	40.64 24.43	Iterations Final SSE DFE MSE S	8 9.45 4 2.36 1.54	a b	48.97 40.64
Solid-to-solvent ratio of 1:30 and 30% ethanol	Iterations Final SSE DFE MSE S	8 10.01 4 2.50 1.58	a b	48.07 12.42	Iterations Final SSE DFE MSE S	7 7.3 4 1.83 1.35	a b	50.45 5.64
Solid-to-solvent ratio of 1:15 and 60% ethanol	Iterations Final SSE DFE MSE S	10 21.92 4 5.48 2.34	a b	42.72 20.11	Iterations Final SSE DFE MSE S	7 16.32 4 4.08 2.02	a b	48.48 19.23
Solid-to-solvent ratio of 1:30 and 60% ethanol	Iterations Final SSE DFE MSE S	7 2.89 4 0.72 0.85	a b	45.50 5.91	Iterations Final SSE DFE MSE S	8 18.35 4 4.59 2.14	a b	59.47 6.27

SSE – Sum of Squares for Error
DFE – Degrees of Freedom for Error
MSE – Mean Squared Error
S – Standard Deviation. The coefficients a and b of the equation and the divergence of the actual from the theoretical values were estimated based on data processing in MINITAB 21.

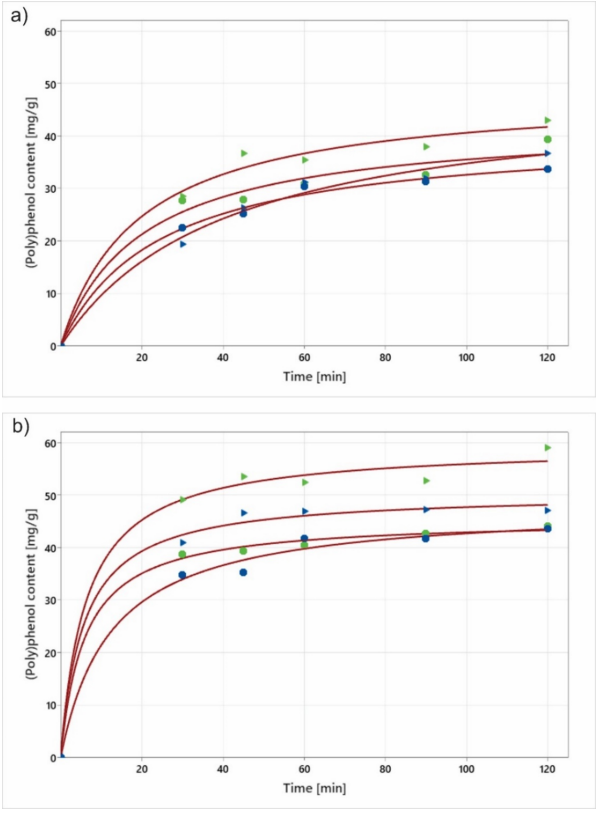

Figure 1. Effect of extraction time on the content of extracted total (poly)phenols at a) solid-to-solvent ratio of 1:15 w/v and b) solid-to-solvent ratio of 1:30 w/v (Blue triangle - UAE and 30% ethanol, Green triangle - UAE and 60% ethanol, Blue circle – no mixing and 30% ethanol and Green circle – no mixing and 60% ethanol).

60% ethanol. The initial extraction is much faster, and in it 49.26 mg GAE/g of (poly)phenols is extracted from the sample, while with further extension of the extraction time, the (poly)phenols content increased to 59.18 mg GAE/g, which represents an increase of only 16.76%.

When using the solid-to-solvent ratio of 1:15 w/v and 60% ethanol, in the second period of extraction there is an increase of (poly)phenols in the extract in the amount of 33.96% (from 28.48 mg GAE/g to 43.13 mg GAE/g), while when using the solid-to-solvent ratio of 1:30 w/v and 30% ethanol the yield (poly)phenols is only 12.92% (from 41.03 mg GAE/g to 47.12 mg GAE/g). Therefore, it can be concluded that using the solid-to-solvent ratio of 1:15 w/v, the extraction is somewhat slower at the beginning, but does not slow down drastically after the initial period, as is the case with higher solid-to-solvent ratios.

Analyzing the influence of the solid-to-solvent ratio at a certain point on the diagram, it is observed that this ratio has a great influence on the yield of (poly)phenols in the extract. For example, during ultrasound-assisted extraction and after 45 min of extraction, when using solvent with 30% ethanol, the content of (poly)phenols at the solid-to-solvent ratio of 1:30 w/v (46.64 mg GAE/g) is higher by 43.38% compared to solid-to-solvent ratio of 1:15 w/v (26.41 mg GAE/g), while when using solvent which contants 60% of ethanol, the content of (poly)phenols at the solid-to-solvent ratio of 1:30 w/v (53.58 mg GAE/g)is higher by 31.26% compared to solid-to-solvent ratio of 1:15 w/v (36.83 mg GAE/g). Therefore, by using a smaller amount of drug (solid substance), it is possible to achieve higher yields of (poly)phenols in the extract. These results were consistent with mass transfer principles where the driving force for mass transfer is considered to be the concentration gradient between the solid and the solvent (Predescu et al. 2016; Tan, Tan, & Ho 2011). Higher solid-to-solvent ratio increases the concentration gradient, leading to an increased diffusion rate of the compounds from the extracted solid material into the solvent, but also determines the increasing of the necessary period of time to achieve equilibrium. Solid-to-solvent ratio could significantly affect the equilibrium constant and characterize the relationship between yield and solvent use as a steep exponential increase followed by a steady state to give the maximum yield (Hamdan, Daood, Toth-Markus, & Illés 2008; Predescu et al. 2016).

The ethanol content in the solvent has a different effect on the (poly)phenols yield depending on the solid-to-solvent ratio. For example, during ultrasound-assisted extraction, after 45 min of extraction and a solid-to-solvent ratio of 1:15 w/v, the yield of (poly)phenols is even 28.29% higher when using 60% ethanol (36.83 mg GAE/g) compared to 30% ethanol (26.41 mg GAE/g), while with the solid-to-solvent of 1:30 w/v yield of

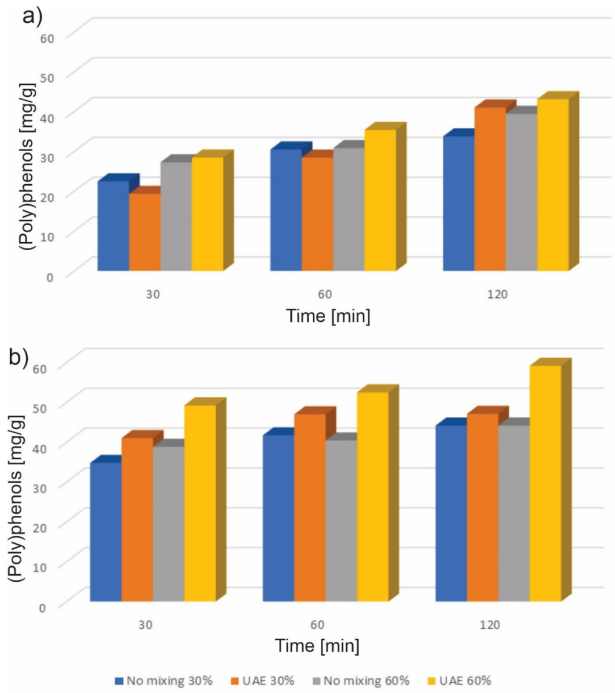

Figure 2. Dependence of (poly)phenol content on time at a) solid-to-solvent ratio of 1:15 w/v and b) solid-to-solvent ratio of 1:30 w/v.

(poly)phenols is higher by only 12.95% (53.58 mg GAE/g with 60% ethanol and 46.64 mg GAE/g with 30% ethanol).

Figure 2 shows the dependence of (poly)phenol yield for different solid-to-solvent ratios at three different extraction times.

It can be seen from the previous diagrams that reaching the plateau, where the concentration of (poly)phenols in the solution almost does not change, depends greatly on the process conditions under which the extraction is performed. First of all, comparing different solid-to-solvent ratios, it is noticed that at their lower ratios the plateau is reached in 60 min, while at higher solid-to-solvent ratios the plateau is reached in a much shorter time. Also, comparing the UAE with the extraction performed without stirring (e.g. with a solid-to-solvent ratio of 1:30 and the use of 60% ethanol), it is noticed that with the extraction without stirring a plateau is reached already in the first 30 minutes, while with UAE extraction continues with further time extension. On the other hand, at the same ratio but with the use of 30% ethanol, it is observed that a plateau is reached already in the first 30 minutes, regardless of the type of mixing.

The results of the ANOVA analysis for determination of flavonoids in extract are shown in Table 3.

Figure 3 shows the dependence of extracted flavonoids on time at constant other process conditions (solid-to-solvent ratio and ethanol content in the sol-

Table 3. Determination of coefficients a and b in equation Y=aX/(b+X) for flavonoids content

Constant values	Conventional extraction (without mixing)				Ultrasound-assisted extraction			
	Summary		Coefficients		Summary		Coefficients	
Solid-to-solvent ratio of 1:15 and 30% ethanol	Iterations Final SSE DFE MSE S	8 7.26 4 1.81 1.35	a b	25.38 39.81	Iterations Final SSE DFE MSE S	9 4.54 4 1.13 1.06	a b	32.18 39.32
Solid-to-solvent ratio of 1:30 and 30% ethanol	Iterations Final SSE DFE MSE S	6 0.41 4 0.10 0.32	a b	18.26 7.03	Iterations Final SSE DFE MSE S	8 18.04 4 4.52 2.12	a b	31.70 15.87
Solid-to-solvent ratio of 1:15 and 60% ethanol	Iterations Final SSE DFE MSE S	9 2.49 4 0.62 0.79	a b	22.28 14.43	Iterations Final SSE DFE MSE S	9 15.79 4 3.94 1.99	a b	45.34 32.18
Solid-to-solvent ratio of 1:30 and 60% ethanol	Iterations Final SSE DFE MSE S	8 1.04 4 0.26 0.51	a b	20.25 12.17	Iterations Final SSE DFE MSE S	8 18.85 4 4.71 2.17	a b	39.12 7.07

vent). As in the case of the dependence of (poly)phenols on time, the curves for extraction without mixing and UAE are given in each diagram. Analyzing the content of flavonoids from Figure 3, it can be seen that their content represents 50% of the content of (poly)phenols during conventional extraction, and 60% of the content of (poly)phenols extracted with UAE. Therefore, it can be concluded that a large proportion of (poly)phenolic compounds are flavonoids in the black elderberry flower.

Similar to the extraction of total (poly)phenols, there are two periods of extraction: initial (fast) extraction and stable (slow) extraction. However, the velocity of extraction in those two different periods depends on the process parameters. In ultrasonic extraction, it is observed that the extraction rate does not stagnate significantly in the slow extraction period. Thus, with a solid-to solvent ratio of 1:15 w/v and 30% ethanol, the yield increases by 46.6% (from 12.49 mg CTH/g to 23.4 mg CTH/g), with a solid-to solvent ratio of 1:15 w/v and 60% ethanol, there is an increase in yield by 45% (from 19.58 mg CTH/g to 35.85 mg CTH/g), and with a solid-to solvent ratio of 1:30 w/v and 30% ethanol yield of flavonoids increases by 33% (from 19.00 mg CTH/g to 28.38 mg CTH/g). The only exception is the solid-to solvent ratio of 1:30 w/v and the use of 60% ethanol, where the yield was increased by only 16% in the period of slow extraction (from 31.68 mg CTH/g to 37.78 mg CTH/g).

In the case of UAE, the maximum yield after 120 minutes of extraction achieved at the solid-to solvent ratio of 1:30 w/v and 60% ethanol is 37.78 mg CTH/g. Also, a favorable yield is achieved with the same ethanol content

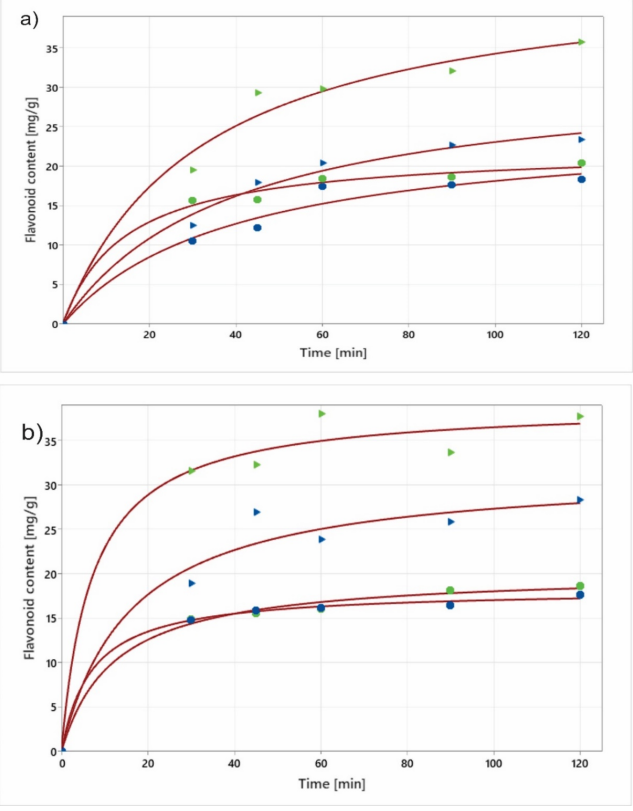

Figure 3. Effect of extraction time on the content of extracted flavonoids at a) solid-to-solvent ratio of 1:15 w/v and b) solid-to-solvent ratio of 1:30 w/v (Blue triangle - UAE and 30% ethanol, Green triangle - UAE and 60% ethanol, Blue circle – no mixing and 30% ethanol and Green circle – no mixing and 60% ethanol)

and the solid-to solvent ratio of 1:15 w/v, which is only 5% lower than the achieved maximum yield.

Looking at one point in time, e.g. after 45 min of extraction, with UAE, a similar effect is observed as with (poly)phenols yield - more efficient extraction is achieved at higher solid-to-solvent ratios and higher ethanol content in the solvent. With the use of 30% ethanol, at a higher solid-to-solvent ratio, it is possible to achieve up to 33.33% higher yield (17.98 mg CTH/g with solid-to-solvent ratio of 1:15 w/v and 26.96 mg CTH/g with solid-to-solvent ratio of 1:30 w/v). This increase is not so pronounced with 60% ethanol, where the yield is only 8.14% (29.4 mg CTH/g with solid-to-solvent ratio of 1:15 w/v and 32.36 mg CTH/g with solid-to-solvent ratio of 1:30 w/v).

Figure 4 shows the dependence of the yield of flavonoids for different solid-to-solvent ratios at three different extraction times.

From the Figure 4, it can first be seen that UAE has a much greater impact on the extraction of flavonoids compared to extraction without mixing. Comparing different solid-to-solvent ratios when using 60% ethanol, it is noticed that the UAE reaches a plateau already in the first 30 minutes at higher solid-to-solvent ratios, while at lower solid-to-solvent ratios there is no clearly discernible plateau. On the other hand, with the use of extraction without stirring, a plateau is reached already in the first 30 minutes, independent of other process conditions. Comparing UAE using 30% ethanol with non-stirred extraction using both levels of ethanol content in the solvent, it is observed that extraction of flavonoids by UAE is much more intense using higher solid-to-solvent ratios than at lower solid-to-solvent ratios.

The results of the ANOVA analysis for determination of anthocyanins in extract are shown in Table 4.

Figure 5 shows the dependence of extracted anthocyanins on time at constant other process conditions (solid-to-solvent ratio and ethanol content in the solvent).

The content of anthocyanins is much lower compared to the total content of (poly)phenolic compounds, which means that used extraction with ethanol is not effective for the extraction of these compounds. The maximum value of anthocyanins is only 0.972 mg Cy3G/g. As in the previous cases, the yield is higher with the use of UAE than with the use of conventional extraction. Also, the diagrams show a clearer difference between extraction without mixing and ultrasonic extraction with the use of a solvent of higher concentration.

Also, it is noticeable that much higher yields are achieved with the use solvent with higher concentration of ethanol, regardless of the type of extraction. Thus, after 120 min of ultrasound-assisted extraction, using a ratio of solid-to-solvent ratio of 1:15 w/v the yield is 5.5

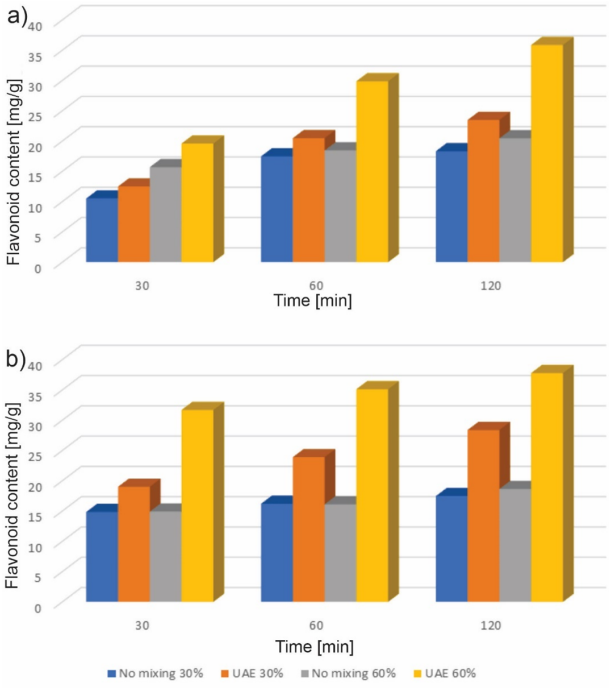

Figure 4. Dependence of flavonoid content on time at a) solid-to-solvent ratio of 1:15 w/v and b) solid-to-solvent ratio of 1:30 w/v.

times higher with the solvent of 60% ethanol (0.712 mg Cy3G/g) compared to solvent of 30% ethanol (0.126 mg Cy3g/g), while with a ratio of solid-to-solvent ratio of 1:30 w/v, the yield is over 4 times higher with use of solvent with higher ethanol content (0.972 mg Cy3G/g with 60% ethanol compare to 0.228 mg Cy3G/g with 30% ethanol). Increasing the solid-to-solvent ratio, increases the anthocyanins content in the extract increases, but the influence of this parameter is not so pronounced.

Figure 6 shows the dependence of anthocyanin yield for different solid-to-solvent ratios at three different extraction times.

From the previous diagrams, it can be seen that the use of 30% ethanol gives very low yields regardless of the solid-to-solvent ratio and the type of mixing, which means that for the extraction of anthocyanins it is necessary to use a solvent with a high ethanol content. On the other hand, it is noticed that when using a solid-to-solvent ratio of 1:15 w/v, the type of mixing does not have a great influence on the extraction, that is, that favorable yields can be achieved without mixing. With extraction without stirring, a plateau with a constant anthocyanins content is reached already at 30 min, while this effect is not so pronounced with the UAE, especially at higher solid-to-solvent ratios.

Table 4. Determination of coefficients a and b in equations Y=aX/(b+X) for anthocyanins content.

Constant values	Conventional extraction (without mixing)				Ultrasound-assisted extraction			
	Summary		Coefficients		Summary		Coefficients	
Solid-to-solvent ratio of 1:15 and 30% ethanol	Iterations Final SSE DFE MSE S	8 $1.5 \cdot 10^{-5}$ 4 $3.8 \cdot 10^{-6}$ $1.9 \cdot 10^{-3}$	a b	0.0987 26.79	Iterations Final SSE DFE MSE S	8 $8.5 \cdot 10^{-4}$ 4 $2.1 \cdot 10^{-4}$ $1.4 \cdot 10^{-2}$	a b	0.1623 35.09
Solid-to-solvent ratio of 1:30 and 30% ethanol	Iterations Final SSE DFE MSE S	8 $5.5 \cdot 10^{-4}$ 4 $1.3 \cdot 10^{-4}$ $1.1 \cdot 10^{-2}$	a b	0.2899 64.65	Iterations Final SSE DFE MSE S	10 $5.5 \cdot 10^{-4}$ 4 $1.4 \cdot 10^{-4}$ $1.1 \cdot 10^{-2}$	a b	0.3831 89.73
Solid-to-solvent ratio of 1:15 and 60% ethanol	Iterations Final SSE DFE MSE S	10 $9.6 \cdot 10^{-3}$ 4 $2.4 \cdot 10^{-3}$ $4.9 \cdot 10^{-2}$	a b	0.6704 23.54	Iterations Final SSE DFE MSE S	9 $1.8 \cdot 10^{-3}$ 4 $4.7 \cdot 10^{-4}$ $2.2 \cdot 10^{-2}$	a b	0.8463 26.28
Solid-to-solvent ratio of 1:30 and 60% ethanol	Iterations Final SSE DFE MSE S	7 $1.2 \cdot 10^{-3}$ 4 $3.0 \cdot 10^{-4}$ $1.7 \cdot 10^{-2}$	a b	0.7499 18.25	Iterations Final SSE DFE MSE S	10 $1.5 \cdot 10^{-2}$ 4 $3.8 \cdot 10^{-3}$ $6.1 \cdot 10^{-2}$	a b	1.2204 36.75

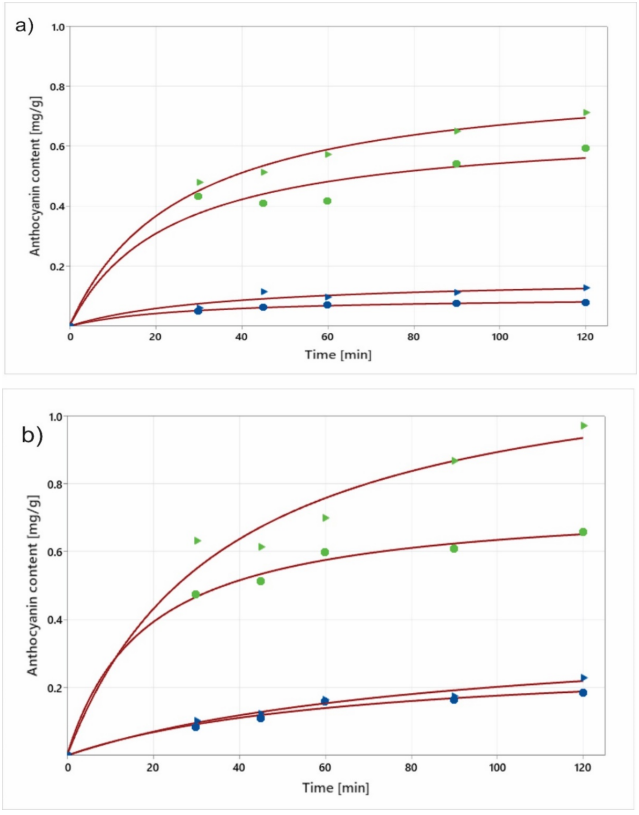

Figure 5. Effect of extraction time on the content of extracted anthocyanins at a) solid-to-solvent ratio of 1:15 w/v and b) solid-to-solvent ratio of 1:30 w/v (Blue triangle - UAE and 30% ethanol, Green triangle - UAE and 60% ethanol, Blue circle – no mixing and 30% ethanol and Green circle – no mixing and 60% ethanol)

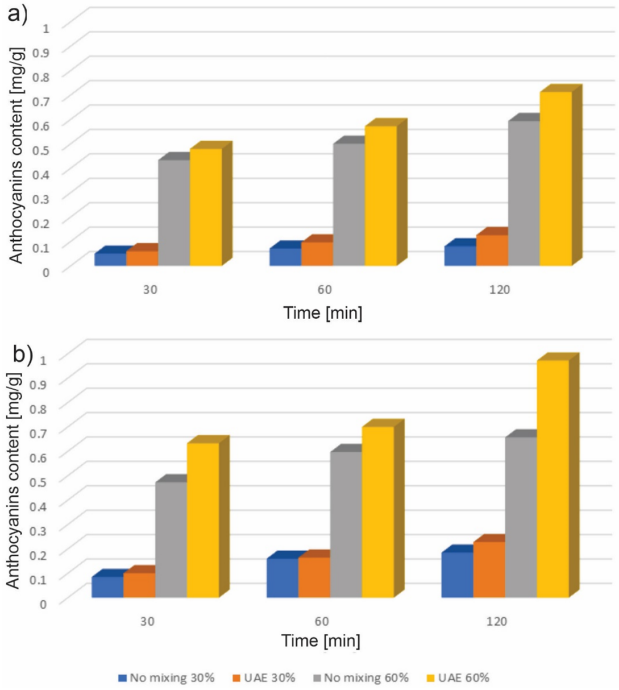

Figure 6. Dependence of anthocyanin content on time at a) solid-to-solvent ratio of 1:15 w/v and b) solid-to-solvent ratio of 1:30 w/v.

4. CONCLUSION

Time as a process parameter was found to have a substantial influence on (poly)phenol yield. At lower solid-to-solvent ratios, the plateau is reached in 60 min, while at higher solid-to-solvent ratios, the plateau is reached in 30 min. Furthermore, with extraction without mixing, the plateau is reached in the first 30 minutes, while with the UAE, the extraction continues with a further extension of time. By examining the effect of varying ethanol concentration in the solvent on (poly)phenols yields, it was found that as the ethanol content increases in solvent, the yield also increases. In terms of the solid-to-solvent ratio influence, greater ratios contribute to more efficient (poly)phenolic compound separation due to easier diffusion of (poly)phenols compounds into the liquid. By comparing the data for the content of (poly)phenols and flavonoids in the extract, it is inferred that flavonoids account for approximately half of the (poly)phenolic components. Unlike that, the content of anthocyanins is much lower compared to the total content of (poly)phenolic compounds. Also, it was discovered that a higher ethanol content in the solvent promote anthocyanins extraction. Comparing classical extraction and ultrasound-assisted extraction, it was determined that ultrasound extraction contributes to a better extraction of (poly)phenolic compounds. Moreover, their difference is clearer when higher solid-to-solvent ratios and solvent with higher ethanol content are used.In future research, it is necessary to optimize the process parameters using a statistical program in order for maximizing the yield of (poly)phenols.

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