Optimization of Total Monomeric Anthocyanin and Total Flavonoid Content Extractions from Purple Potato Tubers Using Ultrasonic Treatments

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The recent interest on phenolic bioactive compounds has caused an increased attention for optimizing the techniques used in bioactive phytochemicals extraction from different natural sources. In order to determine the best extraction conditions using direct sonication were varied amplitude (20, 50 and 80%) ant sonication time (5, 15 and 25 min). To optimize the extraction process was performed mathematical modeling (full factorial experiments 2^2) using SigmaXL software. The total anthocyanins content were determined spectrophotometrically by the pH differential method and the total flavonoids content were determine colorimetric by $AlCl_3$ method.

Keywords: purple potato tuber, anthocyanin pigments, flavonoids, sonication

Potato is the fourth food culture of the world, after corn, wheat and rice, with a production of 329 million tonnes per year. Worldwide, in terms of harvested area potato ranks seven after wheat, rice, corn, barley, sorghum and rapeseed. In terms of consumption, potato ranks third after rice and wheat. In Romania, currently, from the total cultivated area of 8.9 million hectares, potato ranks third with a share of about 3.2% after cereals which represent 62% and oilseeds 15% [1].

Potatoes are significant source of natural antioxidants and exhibit antioxidant activity as demonstrated in recent time by many authors. Studies have indicated that these phytochemicals have high free-radical scavenging activity, which helps to reduce the risk of chronic diseases and age-related neuronal degeneration [2]. Genotypes of potato with peel and pulp intensely colored (red, purple, blue) have antioxidant capacity 2-3 times higher than the white / yellow genotypes, and these aliments could help to supplement the required daily doses of antioxidants in the diet [3]. As a result, in recent years, breeder's efforts intensified to get new potato genotypes in different versions: blue peel and pulp [4, 5].

Because anthocyanin pigments are powerful antioxidants [6] has increased the need to improve extraction techniques. In order to increase the extraction efficiency can be used ultrasonic extraction technique. Ultrasound-assisted extraction is one of the most important techniques used for the extraction of valuable compounds from plant materials and is quite adaptable to a small or large scale. The method involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz, which increases the permeability of cell walls and causing cell lysis, thus favoring the extraction of biologically active compounds [7, 8]. The application of ultrasound-assisted extraction for plant materials [9] and its opportunities in the food processing industry [10] has proved many advantages [11].

The main objective of this paper was to optimise the extraction yield of total anthocyanin and total flavonoid content from purple potato tuber by varying the amplitude and sonication time of a direct ultrasonic treatment. The ultrasonic conditions were compared with an indirect sonication using a bath system.

Experimental part

Material and methods

Plant materials

The potato variety, violet-blue de Galanesti, a population found in Romania [10], was analysed after 20 days of harvest from the research field of NIRDPSB Brasov, Romania.

Sample preparation

Purple potato in amount of 0.5 g (±0.02 g) was homogenized in 10 mlLof 1% acidified water or 1% acidified ethanol. The sample was treated with ultrasonic waves (UP400S, Hielscher USA, Inc) using an ultrasonic probe with a 0.7 cm diameter cylindrical titanium alloy head operated at 24 kHz and 400 W (fig. 1). The tip of the probe was placed at 0.2 cm above the sample mixture and treated following each condition presented in table 1. After the ultrasonic treatment, the sample mixture was centrifuged (10000 rpm, 15 min) and concentrated at 45°C. All the experiments were conducted in triplicate, the results are expressed as mean value ± standard deviation and for significant difference on $p < 0.05$.

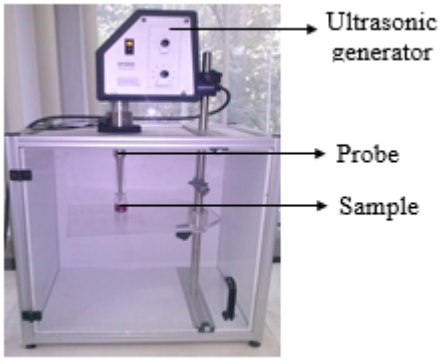

Fig. 1. Experimental setup for direct sonication

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Sample	1	2	3	4	5	6	7	8	9
Amplitude (%) of the maximum amplitude	20			50			80		
Frequncy (kHz)	24								
Time (min)	5	15	25	5	15	25	5	15	25

Table 1
CONDITIONS APPLIED FOR TAC AND TFC EXTRACTION USING DIRECT SONICATION

Factor	Level 1	Level 2	Middle point
T: Sonication time (min)	5	25	15
A: Amplitude (%)	20	80	50

Table 2
LEVELS IN FULL FACTORIAL EXPERIMENTS FOR DIRECT SONICATION

Concentration (mg/mL)	0.005	0.01	0.02	0.025	0.03	0.04	0.05
Absorbance (510nm)	0.065	0.125	0.231	0.274	0.322	0.436	0.547

Table 3
ABSORBANCE AT 510nm OF DIFFERENT CONCENTRATION OF QUERCETINE

Full factorial design for the extraction process of anthocyanins and flavonoids

A two-level and two-factor full factorial experiment (table 2) with two-centered point was design and created using SigmaXL statistical analysis software (Econotron Software Inc., Canada) to cover the range of investigated ultrasonic treatment time and amplitude. Sonication time and sonication amplitude were chosen as independent variables ant the total flavonoid content (TFC) and total monomeric anthocyanins (TAC) were the responses of the design.

The full factorial experimental design for each response with each extraction solvent was analyzed using SigmaXL and expressed in a first order model:

$$Y = \beta_0 + \beta_1 T + \beta_2 A + \beta_{12} TA \quad (1)$$

The semnifications of symbols used in this formula are:
Y – Response design (TFC or TAC);
β_0 – Intercept term;
β_1 and β_2 – Linear coefficients;
β_{12} – Interaction coefficient;
T and A – Independent variables (sonication time and amplitude).

Determination of total monomeric anthocyanin content

The total monomeric anthocyanins content (TAC) were determined through pH differential method [11] based on the property of anthocyanin pigments to change the color with *p*H. Two dilutions of the same sample were prepared, the first one in potassium chloride buffer (0.025 M, *p*H 1.0) and the second one in sodium acetate buffer (0.4 M, *p*H 4.5), *p*H being adjusted with HCl 0.2N. After equilibration at room temperature for 15 min, the absorbance of two dilutions was read at 510 nm and 700 nm using a UV-Vis Microplate Readers (Sunrise-Basic Tecan, Switzerland). Total monomeric anthocyanins - mg cyanidin 3-glucoside (cy-3-glu) equivalent / 100 g Fresh Weight - were calculated as follows:

$$\%\, w/w = \frac{A}{\varepsilon L} MW \; DF \; \frac{V}{W_t} \; 100 \quad (2)$$

$$A = \left(A_{510nm} - A_{700nm}\right)_{pH=1} - \left(A_{510nm} - A_{700nm}\right)_{pH=4.5} \quad (3)$$

The semnifications of symbols used in these relations are:
A – Absorbance
ε – Molar extinction coefficient (34300 L / mol . cm for cy-3-glu)
L – Path length
MW – Molecular weight (484.84 g/mol for cy-3-glu)
DF – Dilution factor
V – Volume
W_t – sample weight

Determination of total flavonoid content

The total flavonoid content (TFC) of purple potato extracts was determined by a colorimetric method as described previously in other studies [12, 13].

The extracts were diluted with 2 mL of distilled water and 150 µL 5% $NaNO_2$ was added. After 6 min the mixture was treated with 150 µL $AlCl_3$ 10% and, after 6 min, with 2 ml NaOH 1N and the volum were made to 5 mL. The absorbance was recorded at 510 nm using a specto-photometer (DR2800, Hach, USA) and the flavonoid content was expressed as mg of quercetin equivalents for 100 g of Fresh Weigh (FW).

For building the calibration curve, quercetine is used as a standard materials. Various concentrations (table 3) of standard quercetine solution were used to make a standard calibration curve (fig. 2).

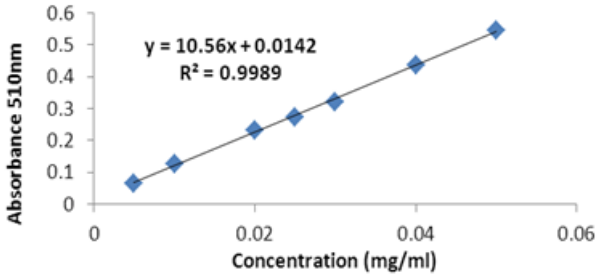


Fig. 2. Calibration curve of quercetine

Concentration values of extracts were obtained from quercetine standard curve, by interpolating to the X- axis. TFC was calculated by using the following formula:

$$TFC = (R \,.\, DF \,.\, V \,.\, 100) / W \quad (3)$$

The semnifications of symbols used in this relation are:
R - Result obtained from the standard curve;
DF - Dilution factor;
V – Volume;
W – Sample weight.

Comparison between direct and indirect sonication

The results from direct sonication were compared to an indirect ultrasonic bath system (fig. 3) where the ultrasonic generator is powered at 240 W with operating frequency of 35 kHz. In case of indirect sonication ultrasound is transmitted through a fluid compared to direct sonication where the ultrasonic probe is immersed directly in the sample mixture. The controls are simple extraction in solvent with intermittent shaking. All the experiments including controls were conducted in triplicate, the results are expressed as mean value ± standard deviation and for significant difference on $p < 0.05$.

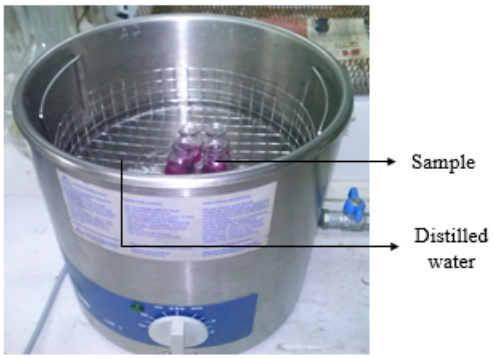


Fig. 3. Experimental setup for indirect sonication

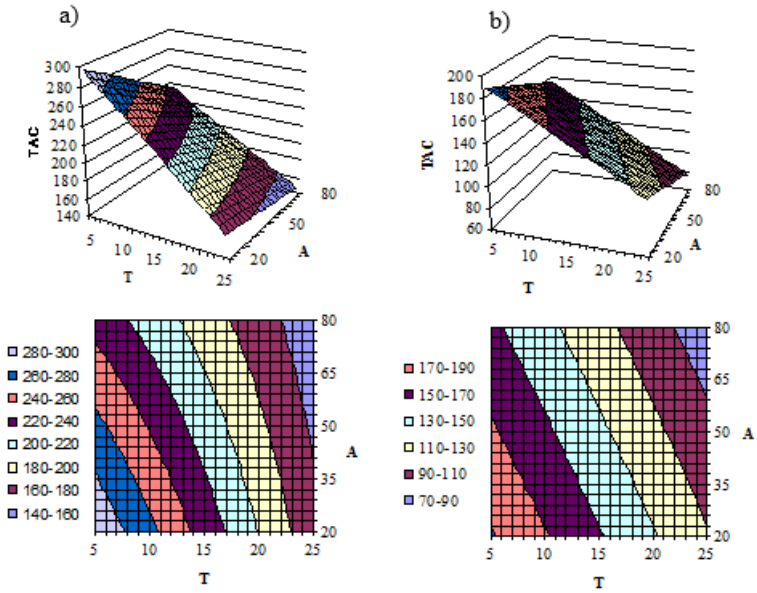


Fig. 4. Response contur plot and surface plot showing the effect of sonication time - T (min) and amplitude - A (%) on TAC extraction from purple potato tuber: a) 1% acidified water, b) 1% acidified ethanol

Run order	T (min)	A (%)	TAC (mg cy-3-glu/100 g FW)				Residuals for water	Residuals for ethanol
			Experimental values		Predicted values			
			Water	Ethanol	Water	Ethanol		
1*	5	20	**297.72**	**193.01**	**298.01**	**190.21**	-0.29	2.80
2	25	20	167.54	110.33	166.31	112.44	1.23	-2.11
3	5	80	235.29	155.12	233.34	154.65	1.95	0.47
4	25	80	148.12	76.41	147.00	76.88	1.12	-0.47
5	5	20	295.65	190.98	298.01	190.21	-2.35	0.77
6	25	20	162.44	113.25	166.31	112.44	-3.87	0.81
7	5	80	228.75	152.87	233.34	154.65	-4.59	-1.78
8	25	80	143.23	80.93	147.00	76.88	-3.76	4.05
9	15	50	213.76	132.56	211.16	133.54	2.60	-0.98
10	15	50	219.14	129.98	211.16	133.54	7.98	-3.56

* Optimum conditions for TAC extraction for both solvents, 1% acidified water and 1% acidified ethanol.

Table 4
EFFECT OF SONICATION TIME AND AMPLITUDE ON TAC EXTRACTION FROM PURPLE POTATO TUBER USING FULL FACTORIAL DESIG

Results and discussions

Optimization of sonication time and amplitude for TAC

In the table 4 can be observed that the maximum TAC of 297.72 ± 3.63 mg cy-3-glu/100 g FW was obtained at 20% amplitude using 1% acidified water, which was 35.17% significantly higher than the maximum TAC of 193.01 ± 4.83 mg cy-3-glu /100 g FW using 1% acidified ethanol at the same amplitude and sonication time of 5 min.

The models are well fitted with linear regression equations for both water (TAC_W) and ethanol (TAC_E) with high values of determinations coefficient and low residuals (table 4), also observed in the response surface analysis obtained (fig. 4).

$TAC_W = 211.16 - 54.51T - 20.99A + 11.34TA$, $R^2=99.53\%$
$TAC_E = 133.54 - 38.88T - 17.78A$, $R^2=99.68$

For both models all independent variables (sonication time and amplitude) have significant ($p<0.05$) effect on TAC extraction, while interaction between sonication time and amplitude was significant just for first model (TAC_W) and was not significant for second model (TAC_E), because of that was remove from design. For both models (TAC_W and TAC_E), sonication time has a higher effect on TAC extraction (coefficient –54.51 and –38.88) than amplitude (coefficient –20.99 and –17.78).

The optimisation procedure was conducted in order to maximise the total anthocyanins content. Through this models was found that the maximum anthocyanins content was obtained at amplitude of 20% and sonication time of 2.78 min using 1% acidified water (312.625 mg cy-3-glu/100 g FW) and at amplitude of 20% and sonication time of 2.17 min (201.210 mg cy-3-glu/100 g FW) using 1% acidified ethanol.

Optimization of sonication time and amplitude for TFC

In the table 5 can be observed that the maximum TFC of 216.67 ± 5.54 mg quercetin/ 100 g FW was obtained at 80% amplitude using 1% acidified water, which was 48.85% significantly higher than the maximum TFC of 110.83 ± 3.15 mg quercetin / 100 g FW using 1% acidified ethanol at 20% amplitude with the same sonication time of 5 min.

The models are well fitted with linear regression equations for both water (TFC_W) and ethanol (TFC_E) with high values of determinations coefficient and low residuals (table 5), also observed in the response surface analysis obtained (fig. 5).

$TFC_W = 167.06 - 24.73T + 13.26A - 7.81TA$, $R^2=98.75\%$
$TFC_E = 94.93 - 8.43T - 14.13A - 7.47TA$, $R^2=95.80\%$

For both models all factors (sonication time and amplitude) and the interaction between sonication time and amplitude have significant ($p<0.05$) effect on TFC extraction. For first model (TFC_W), sonication time has a higher effect on TFC extraction (coefficient -24.73) than

Run order	T (min)	A (%)	TFC (mg quercetin/100 g FW)				Residuals for water	Residuals for ethanol
			Experimental values		Predicted values			
			Water	Ethanol	Water	Ethanol		
1	5	20	173.78	106.62	170.72	110.02	3.07	-1.07
2	25	20	134.39	105.23	136.87	108.09	-2.49	-0.90
3*	5	80	**216.67**	92.02	**212.85**	96.70	3.81	-1.48
4	25	80	150.62	61.74	147.78	64.91	2.85	-1.00
5**	5	20	168.85	**110.83**	170.72	**110.02**	-1.87	0.26
6	25	20	140.56	108.36	136.87	108.09	3.69	0.09
7	5	80	210.24	98.79	212.85	96.70	-2.61	0.66
8	25	80	146.13	65.48	147.78	64.91	-1.65	0.18
9	15	50	166.84	98.79	167.06	94.93	-0.21	0.93
10	15	50	162.48	101.44	167.06	94.93	-4.58	1.57

* Optimum conditions for TFC extraction using 1% acidified water.
** Optimum conditions for TFC extraction using 1% acidified ethanol.

Table 5
EFFECT OF SONICATION TIME AND AMPLITUDE ON TFC EXTRACTION FROM PURPLE POTATO TUBER USING FULL FACTORIAL DESIGN

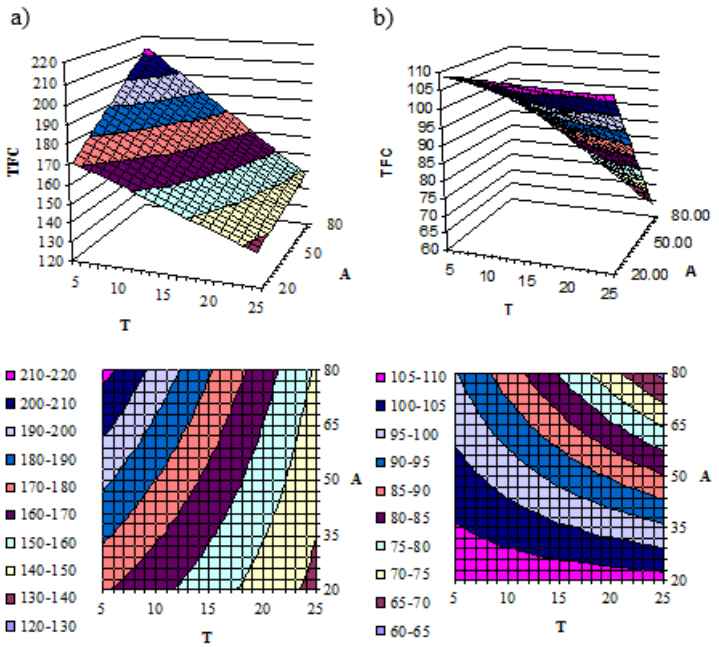
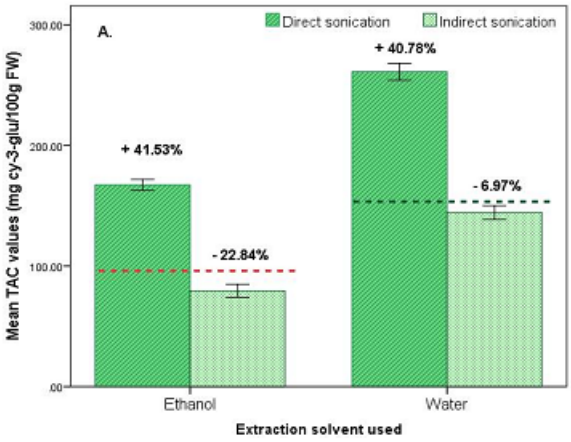


Fig. 5. Response contur plot and surface plot showing the effect of sonication time - T (min) and amplitude - A (%) on TFC extraction from purple potato tuber: a) 1% acidified water, b) 1% acidified ethanol



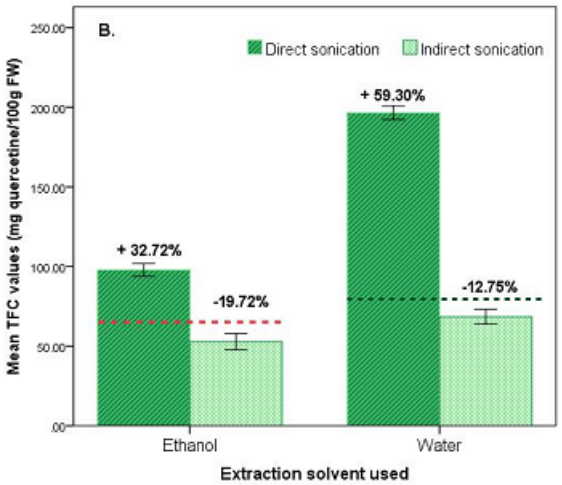


Fig. 6. Comparison of direct and indirect sonication for: A. TAC and B. TFC

amplitude (coefficient +13.26) while for the second model (TFC_E), sonication time has a lower effect on TFC extraction (coefficient –8.43) than amplitude (coefficient -14.13).

The optimisation procedure was conducted in order to maximize the total flavonoids content. Through this models was found that the maximum flavonoids content was obtained at amplitude of 80% and sonication time of 2.65 min using 1% acidified water (220.498 mg quercetine/100g FW) and at amplitude of 20% and sonication time of 2.34 min (110.279 mg quercetine/100g FW) using 1% acidified ethanol.

Comparison of direct sonication and indirect sonication with conventional extraction of TAC and TFC from purple potato tuber

The results of direct sonication (time 5 min and 50% amplitude) were compared with an indirect sonication using a bath and the conventional solvent extraction (sample control). The result of direct sonication for TAC was significantly higher than both the indirect sonication and the control method using 1% acidified water and 1% acidified ethanol as solvent (fig. 6A). For TAC in water using direct sonication, it was a significant increase by 40.78% from control of 154.35 ± 4.05 mg cy-3-glu/100 g FW while using indirect sonication, it was a decrease by 6.97%. Also for TAC in ethanol using direct sonication, it was a significant increase by 41.53% from control of 95.83 ± 6.16 mg cy-3-glu/100 g FW while using indirect sonication, it was a decrease by 22.84%. In comparing both solvents alone, 1% acidified water also gave a higher content of TAC by 35.92% compared to 1% acidified ethanol. The indirect sonication using the ultrasonic bath did not give improvement in the extraction of TAC, on the contrary was observed a significant decrease for both solvent used.

The result of direct sonication for TFC was significantly higher than both the indirect sonication and the control method in 1% acidified water and 1% acidified ethanol (fig. 6B). For TFC in water using direct sonication, it was a significant increase by 59.30% from control of 78.48 ± 2.91 mg quercetin/100 g FW while using indirect sonication, it was a decrease by 12.75%. Also for TFC in ethanol using direct sonication, it was a significant increase by 32.72% from control of 65.83 ± 3.53 mg quercetin/100 g FW while using indirect sonication, it was a decrease by 19.72%. Similarly to the TAC extraction, 1% acidified water also gave a higher content of TFC by 50.22% compared to 1% acidified ethanol. The same like TAC extraction, for the indirect sonication using the ultrasonic bath was observed a significant decrease for both solvents used.

This study proves that direct sonication improves the TAC and TFC extraction compared to indirect sonication and the conventional solvent extraction.

In addition to improved the extraction of bioactive compound from plants, ultrasonic extraction can contribute to reducing the extraction time.

Conclusions

At sonication time of 5 min and 20% amplitude was found giving the highest content of anthocyanins also for 1%acidified water and 1% acidified ethanol, while 5 min and 80% amplitude gave the highest content of flavonoid for 1% acidified water and 5 min and 20% amplitude for 1% acidified ethanol. These conditions using direct sonication have enhanced TAC yields by 40.78% (1% acidified water) and 41.53% (1% acidified ethanol) and also for TFC yields by 59.30% (1% acidified water) and 32.72% (1% acidified ethanol) compared with control.

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