RESEARCH ARTICLE OPEN ACCESS

Total Monomeric Anthocyanin and Total Flavonoid Content of Processed Purple Potato

Florentina Damşa[1,2], Alexandru Woinaroschy[1], Gheorghe Olteanu[2], Carmen Liliana Bădărău[2,3], Angela Mărculescu[3]

[1]"Politehnica" University of Bucharest, Department of Chemical and Biochemical Engineering, 1-7, Polizu Str., 011061, Bucharest, Romania

[2]National Institute of Research and Development for Potato and Sugar Beet, no. 2 Fundăturii street, 500470, Brasov, Romania.

[3]University Transilvania of Brasov, Faculty of Food and Tourism, Brasov, Romania

Abstract

It is well known that processing change physical and chemical composition of foods, thus affecting the content in bioactive substances. Potatoes are almost always consumed after processing (baked, fried or boiled) making it critical to understand the effect of such processing techniques on the containing in bioactive compounds. In order to determine the influence of processing on the content of anthocyanin pigments and flavonoids was achieved the extraction of these compounds from boiled and baked purple potato tuber (Albastru-Violet de Galanesti variety). Also, in order to obtain the maximum amount of anthocyanin pigments and flavonoids from processed potatoes was applied ultrasonic extraction (20 kHz) and was performed the mathematical modeling (central composite design) using SigmaXL software. The total anthocyanins content were determined spectrophotometrically by the pH differential method and the total flavonoids content were determine colorimetric by AlCl3 method. This study proves that the potato processing decreases the content of anthocyanin pigments and flavonoids.

Keywords: purple potato, anthocyanin pigments, flavonoid, processed potato

I. Introduction

Potato is the fourth food culture of the world, after corn, wheat and rice, with a production of 329 million tones 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% (FAO 2012).

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 (Teow et al. 2007). 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 (Damsa et al. 2015). As a result, in recent years, breeder's efforts intensified to get new potato genotypes in different versions: blue peel and pulp (Kosieradzka et al. 2004, Nara et al. 2006).

It is well known that processing changes the physical and chemical composition of foods (Spanos et al. 1990; Price et al. 1997), thus, affecting their antioxidant activity (Nicoli et al. 1999; Dewanto et al. 2002). Potatoes are almost always consumed after processing (baked, chipped, fried, boiled or microwaved) making it critical to understand the effect of such processing techniques on the activity and composition of bioactive compounds in potatoes.

Some authors (Dao and Friedman 1992) reported a 100% loss in the chlorogenic acid content of potatoes baked at 212°C for 45 minutes, which suggested that chlorogenic acid is susceptible to heat. Baking led to an increase in the total phenolic content and antioxidant activity of eight potato genotypes. Samples had greater levels of chlorogenic acid, caffeic acid, *para*-coumaric acid and vanillic acid (Blessington et al. 2010). Baking for 30 minutes increased the total phenolic content and chlorogenic acid content in three potato genotypes. This increase could be due to improvement in the extractability of phenolic compounds as cooking weakens the matrix, and inactivates enzymes that use phenolic compounds as substrate (Ezekiel et al. 2011). The effects of baking cannot be generalized for all potato clones as they differ depending on potato genotype. Researchers found that boiling for 20 minutes did not alter the phenolic acid content but significantly

decreased the anthocyanin content of colored-flesh cultivars (Mulinacci et al. 2008). Researchers reported that boiling for 18 minutes caused an increase in the total phenolic content and chlorogenic acid content in two white-fleshed and one purple-fleshed potato genotypes (Navarre et al. 2010). Boiled samples had greater levels of chlorogenic acid, caffeic acid and vanillic acid (Blessington et al. 2010). A possible reason suggested was the increase in the extractability of phenolic compounds from cooked samples, similar to the observations for baked potato samples.

The optimisation of the extraction of anthocyanins and flavonoids is essential to reach an accurate analysis (Alberti et al. 2013). Response surface methodology (RSM) is an effective tool for optimizing this process. Also, this is a method for improving and optimizing processes, and it can evaluate the effect of the variables and their interactions (Farris and Piergiovanni 2009, Wettasinghe and Shahidi, 1999).

The main objective of this paper was to determine the influence of processing technique on the content of anthocyanin pigments and flavonoids.

II. Materials and methods

2.1. Plant materials

The potato variety, Albastru-Violet de Gălăneşti, a population found in Romania (Morar et al. 2004), was analysed after harvest from the research field of National Institute of Research and Development for Potato and Sugar Beet (NIRDPSB) Braşov, Romania.

2.2. Sample preparation

Purple potato in amount of 4 g (± 0.02 g) was homogenized in 40 ml of 1% acidified water. The extraction was achieved from fresh, baked and boiled purple potato tuber. The sample was treated with ultrasonic waves (UP400S, Hielscher USA, Inc) using an ultrasonic probe with a 1.3 cm diameter cylindrical titanium alloy head operated at 20 kHz and 750 W (Fig. 1). The tip of the probe was placed at 2 cm below 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 $p < 0.05$.

Ultrasonic generator is equipped with a thermocouple and the temperature was set at 40°C to prevent overheating and consequently sample degradation.

Fig. 1. Experimental setup for sonication

Table 1. Conditions applied for anthocyanins and flavonoids extraction from purple potato tuber

Sample	1	2	3	4	5	6	7	8	9
Amplitude (%)	20			50			80		
Frequency (kHz)	20								
Time (min)	5	15	25	5	15	25	5	15	25

2.3. Determination of total monomeric anthocyanin content

The total monomeric anthocyanins content (TAC) were determined through pH differential method (Giusti et al., 2007) based on the property of anthocyanin pigments to change the color with pH. Two dilutions of the same sample were prepared, the first one in potassium chloride buffer (0.025 M, pH 1.0) and the second one in sodium acetate buffer (0.4 M, pH 4.5), pH 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-galactoside (cy-3-glu) equivalent / 100 g Fresh Weight - were calculated as follows:

$$TAC(mg/L) = (A \cdot MW \cdot DF \cdot V \cdot 100)/\varepsilon \cdot L \cdot Wt \quad (1)$$

$$A = (A_{510nm} - A_{700nm})_{pH=1} - (A_{510nm} - A_{700nm})_{pH=4.5} \quad (2)$$

The semnifications of symbols used in these relations are:

A – Absorbance

ε – Molar extinction coefficient (34300 $L/mol \cdot cm$ 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

2.4. 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 (Kim et al., 2003; Zhishen et al., 1999).

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 volume were made to 5 ml. The absorbance was measured at 510 nm using a spectrophotometer (DR2800, Hach, USA) and the flavonoid content was expressed as mg of quercetine equivalents for 100 g of Fresh Weigh (FW).

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

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

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

The semnifications of symbols used in this relation are:

R - Result obtained from the standard curve;
DF - Dilution factor;
V – Volume;
W – Sample weight.

Table 2 Absorbance at 510nm of different concentration of quercetine

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

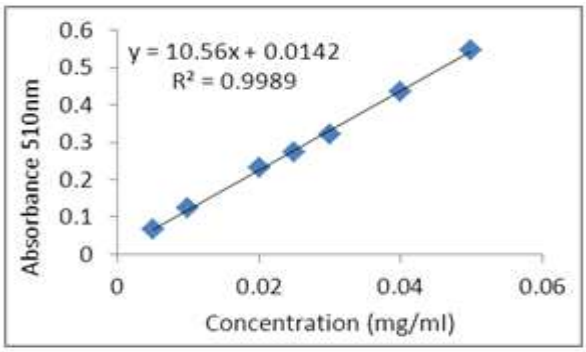

Fig. 2 Calibration curve of quercetine

2.5. Central composite design for the extraction of anthocyanins and flavonoids

Central composite design (CCD) with three-level and two-factor (Table 3) 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 and the total flavonoid content (TFC) and total monomeric anthocyanins (TAC) were the responses of the design.

Table 3. Levels in central composite design

Factor	Level 1	Level 2	Level 3
A: Amplitude (%)	5	15	25
τ: Sonication time (min)	20	50	80

A second-order polynomial equation was used to fit the experimental data of the studied variables. The generalized second-order polynomial model used in the response surface analysis is shown in equation 4.

$$Y=\beta_0 + \beta_1 A + \beta_2 \tau + \beta_{11} A^2 + \beta_{22} \tau^2 + \beta_{12} A\tau \quad (4)$$

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 coefficients;
β_{11} and β_{22} – Quadratic coefficients;
τ and A – Independent variables (sonication time and amplitude).

The statistical significance of the terms in the regression equations was examined by ANOVA for each response. The terms statistically found as non-significant were excluded from the initial model and the experimental data were re-fitted only to the significant ($p < 0.05$) parameters.

III. Results and discussions

3.1. Optimization of sonication time and amplitude for TAC extraction from baked and boiled purple potato tuber

The mean values of the total anthocyanins

content of the extraction performed on purple potato tuber with 1% acidified water are shown in Table 4. The total anthocyanins content ranged from 72.67 mg/100g (run number 4) to 150.863 mg/100g (run number 1) for baked potato and from 85.626 mg/100g (run number 4) to 164.619 mg/100g (run number 1). The highest values for TAC of 164.619 mg cy-3-glu/100 g FW was obtained at 20% amplitude from boiled potato tuber using 1% acidified water, which was higher than the maximum TAC of 150.863 mg cy-3-glu /100 g FW obtained from baked potato tuber using 1% acidified water at the same amplitude and sonication time of 5 min.

The multiple regression analysis of total anthocyanins content values from baked potato showed that the model was significant ($p<0.0001$), did not present lack of fit ($p=0.39$) and it could explain 96.92% of all variance ($R^2_{adj}=0.96$). The predicted model can be described by the equation 5. Interactions coefficient of time (τ) and amplitude (A) increased the anthocyanins extraction, and time (τ), amplitude (A) and quadratic regression coefficient of time (τ) had a significantly negative effect.

$$TAC_C = 118.626 - 18.025A - 23.909\tau + 8.61125A\tau - 16.599\tau^2 \quad (5)$$

The result suggested that the quadratic regression coefficient of amplitude had negligible effects on the extraction of anthocyanins from baked potato.

The multiple regression analysis of total anthocyanins content values from boiled potato showed that the model was significant ($p<0.0003$), did not present lack of fit ($p=0.51$) and it could explain 95.77% of all variance ($R^2_{adj}=0.95$). The predicted model can be described by the equation 6. Interactions coefficient of time (τ) and amplitude (A) increased the anthocyanins extraction, and time (τ), amplitude (A) and quadratic regression coefficient of time (τ) had a significantly negative effect.

$$TAC_F = 125.144 - 18.6A - 20.808\tau + 9.694A\tau - 11.474\tau^2 \quad (6)$$

The models are well fitted with multiple regression equations for both processed technique: baked (TAC_C) and boiled (TAC_F), observed in the response surface analysis obtained (Fig. 3).

Table 4. Effect of sonication time and amplitude on TAC extraction from processed purple potato tuber using central composite design

Run order	τ (min)	A (%)	TAC (mg cy-3-glu/100 g FW)				Residuals for baked	Residuals for boiled
			Experimental values		Predicted values			
			Baked	Boiled	Baked	Boiled		
1*	5	20	**150.863**	**164.619**	152.57	162.77	-1.709	1.847
2	5	80	102.846	111.457	99.299	106.18	3.547	5.274
3	25	20	86.242	100.012	87.532	101.77	-1.290	-1.757
4	25	80	72.67	85.626	68.704	83.956	3.966	1.670
5	15	20	142.862	147.815	136.65	143.74	6.211	4.071
6	15	80	96.299	103.761	100.60	106.54	-4.301	-2.783
7	5	50	124.098	127.357	125.94	134.48	-1.838	-7.121
8	25	50	75.442	92.949	78.118	92.862	-2.676	0.087
9	15	50	115.668	127.357	118.63	125.14	-2.957	2.213
10	15	50	119.673	121.643	118.63	125.14	1.048	-3.501

* Optimum conditions for TAC extraction from baked and boiled potato tuber.

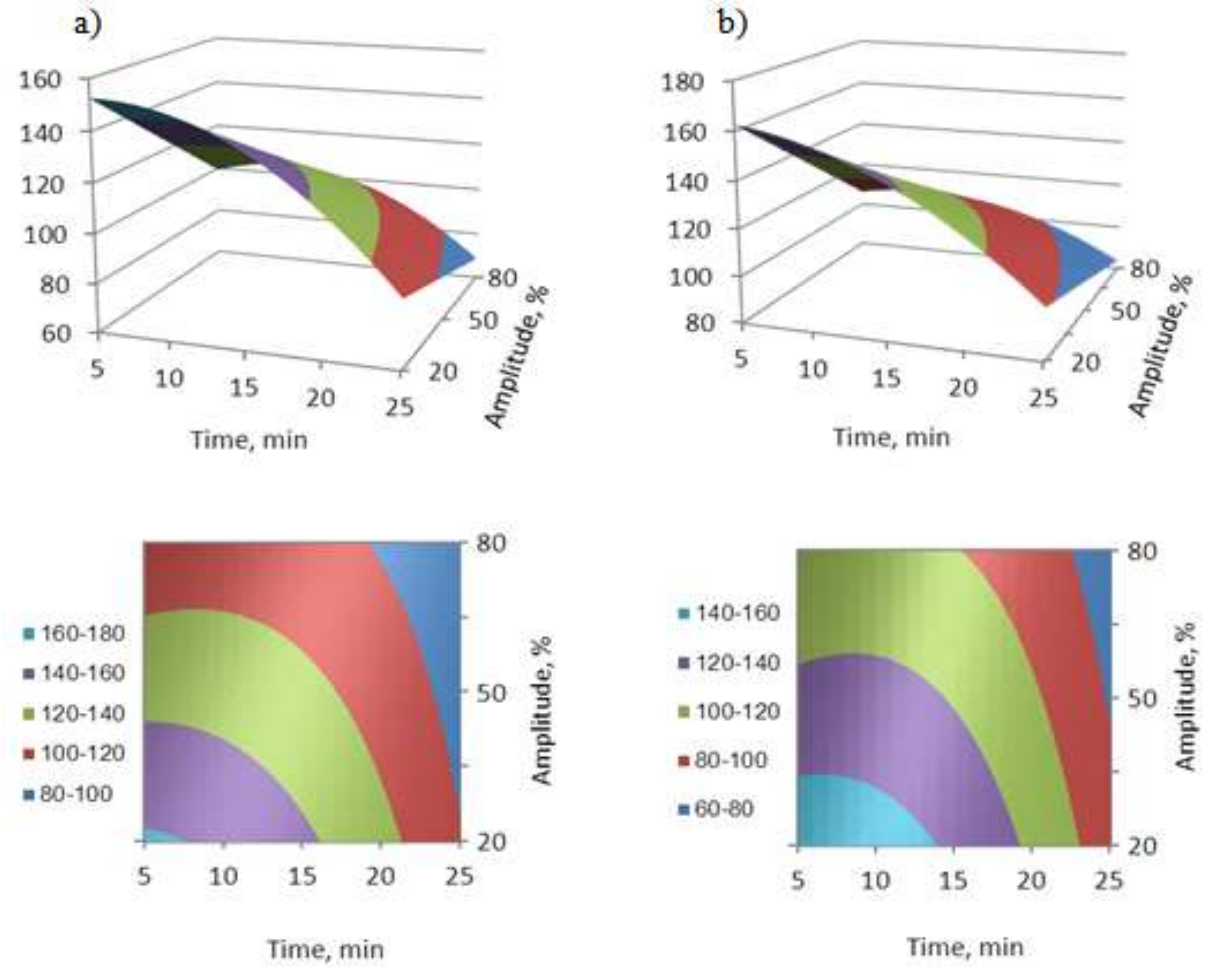

Fig. 3. Response contour plot and surface plot showing the effect of sonication time - τ (min) and amplitude - A (%) on TAC extraction from purple potato tuber: a) baked potato tuber, b) boiled potato tuber.

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 5.20 min for baked potato (152.579 mg cy-3-glu/100 g FW) and, for boiled potato, at amplitude of 20% and sonication time of 1.71 min (164.015 mg cy-3-glu/100 g FW).

3.2. Optimization of sonication time and amplitude for TFC extraction from baked and boiled purple potato tuber

The mean values of the total flavonoids content of the extraction performed on purple potato tuber with 1% acidified water are shown in Table 5. The total flavonoids content ranged from 97.499 mg/100g (run number 4) to 162.852 mg/100g (run number 8) for baked potato and from 98.243 mg/100g (run number 4) to 179.886 mg/100g (run number 8). The highest values for TFC of 179.886 mg cy-3-glu/100 g FW was obtained at 20% amplitude from boiled potato tuber using 1% acidified water, which was higher than the maximum TFC of 162.852 mg cy-3-glu /100 g FW obtained from baked potato tuber using 1% acidified water at the same amplitude and sonication time of 15 min.

The multiple regression analysis of total flavonoids content values from baked potato showed that the model was significant ($p<0.0001$), did not present lack of fit ($p=0.36$) and it could explain 98.19% of all variance ($R^2_{adj}=0.98$). The predicted model can be described by the equation 7. Interactions coefficient of time (τ) and amplitude (A) increased the flavonoids extraction, and time (τ), amplitude (A) and quadratic regression coefficient of time (τ) had a significantly negative effect.

$$TFC_C = 146.692 – 12.005A – 10.876\tau + 3.811A\tau – 30.539\tau^2 \quad (7)$$

The result suggested that the quadratic regression coefficient of amplitude had negligible effects on the extraction of flavonoids from baked potato.

The multiple regression analysis of total flavonoids content values from boiled potato showed that the model was significant ($p<0.0001$), did not present lack of fit ($p=0.71$) and it could explain 98.93% of all variance ($R^2_{adj}=0.98$). The predicted

model can be described by the equation 8. Interactions coefficient of time (τ) and amplitude (A) increased the flavonoids extraction, and time (τ), amplitude (A) and quadratic regression coefficient of time (τ) had a significantly negative effect.

$$TFC_F = 164.698 - 13.794A - 10.131\tau + 4.136A\tau - 47.350\tau^2 \quad (8)$$

The models are well fitted with multiple regression equations for both processed technique: baked (TFC_C) and boiled (TFC_F), observed in the response surface analysis obtained (Fig. 4).

Table 5. Effect of sonication time and amplitude on TFC extraction from processed purple potato tuber using central composite design

Run order	τ (min)	A (%)	TFC (mg quercetin/100 g FW)				Residuals for baked	Residuals for boiled
			Experimental values		Predicted values			
			Baked	Boiled	Baked	Boiled		
1	5	20	142.483	143.164	142.84	145.41	-0.362	-2.245
2	25	50	105.884	108.336	105.28	107.22	0.607	1.118
3	15	50	145.058	168.224	146.69	164.70	-1.633	3.526
4	25	80	97.499	98.243	97.082	97.559	0.417	0.684
5	5	50	126.312	129.483	127.03	127.48	-0.717	2.004
6	25	20	112.447	115.074	113.47	116.88	-1.024	-1.802
7	5	80	112.293	109.789	111.21	109.55	1.079	0.241
8*	15	20	**162.852**	**179.886**	158.70	178.49	4.156	1.393
9	15	50	142.895	163.357	146.69	164.70	-3.796	-1.341
10	15	80	135.961	147.326	134.69	150.90	1.274	-3.578

* Optimum conditions for TFC extraction from baked and boiled potato tuber.

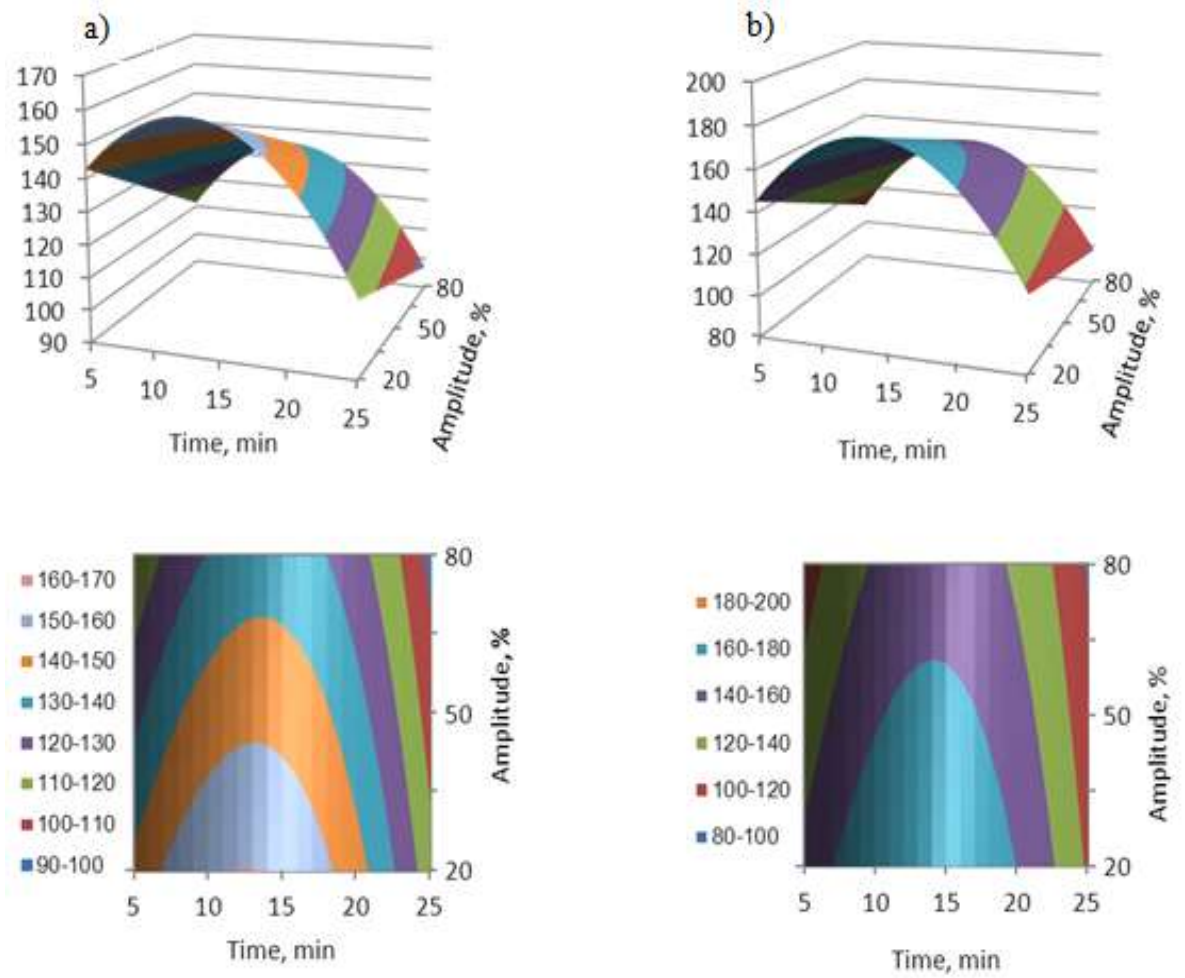

Fig. 4. Response contour plot and surface plot showing the effect of sonication time - τ (min) and amplitude - A (%) on TFC extraction from purple potato tuber: a) baked potato tuber, b) boiled potato tuber.

The optimization 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 20% and sonication time of 12.60 min for baked potato (160.462 mg quercetine/100g FW) and, for boiled potato, at amplitude of 20% and sonication time of 13.49 min (179.567 mg quercetine/100g FW).

3.3. Comparison between extraction of anthocyanins and flavonoids from processed and fresh purple potato tubers

The results of ultrasound extraction from processed potato (amplitude 20% - optimum amplitude for anthocyanins and flavonoid extraction) were compared with the results of ultrasound extraction from fresh potato tuber (sample control).

The result from fresh potato for TAC was significantly higher than both processed technique (baked and boiled) (Fig. 5). For TAC extraction from baked potato was observed a significant decrease that range from 35.99% (sonication time of 15 min.) to 53.44% (sonication time of 25 min). Also, for TAC extraction from boiled potato was observed a significant decrease that range from 33.77% (sonication time of 15 min.) to 46.01% (sonication time of 25 min).

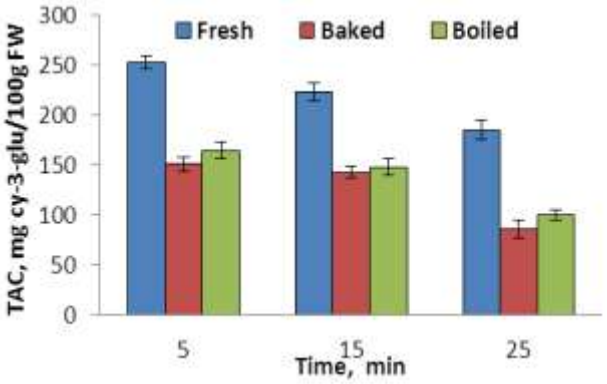

Fig. 5. Influence of processed technique on TAC extraction from potato tuber.

The result from fresh potato for TFC was significantly higher than both processed technique (baked and boiled) (Fig. 6). For TFC extraction from baked potato was observed a significant decrease that range from 21.52% (sonication time of 15 min.) to 33.59% (sonication time of 25 min). Also, for TFC extraction from boiled potato was observed a significant decrease that range from 13.31% (sonication time of 15 min.) to 32.04% (sonication time of 25 min).

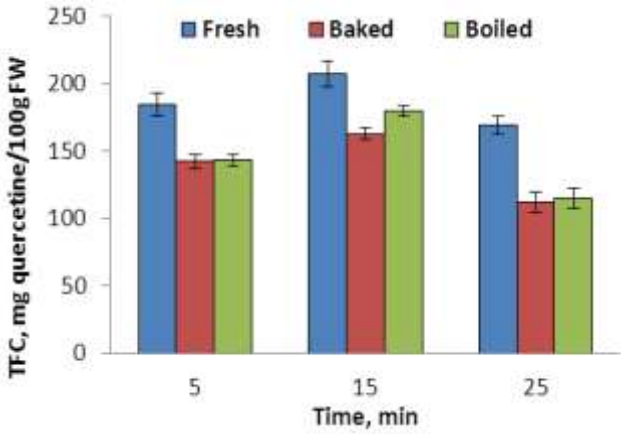

Fig. 6. Influence of processed technique on TFC extraction from potato tuber.

IV. Conclusions

CCD was effective in estimating the effect of two independent variables on the extraction of total anthocyanins and total flavonoids compounds in purple potato tuber. For TAC the best results was obtained at 20% amplitude and 5 min, and for TFC the best results was obtained at 20% amplitude and 15 min. Also, CCD was successfully used to obtain the optimum conditions for TAC and TFC.

For both processing methods was observed a significant decrease in the content of anthocyanins and flavonoids in processing potato.

V. Acknowledgement

This work was partially supported by a grant of the Romanian National Authority for Scientific Research, CNDI-UEFISCDI, PN-II-PT-PCCA-2013-4-0452 (178/2014) and PN-II-PT-PCCA-2013-4-1629 (225/2014).

References

[1.] Aline, A., Acácio, A., Zielinski, F., Zardo, D.M., c, Ivo, M.D., Nogueira A., Mafra, L.I. (2014). Optimisation of the extraction of phenolic compounds from apples using response surface methodology. FOOD CHEM, 149, 151–158.

[2.] Blessington, T., M. Nzaramba, D. Scheuring, A. Hale, L. Reddivari and J. Miller (2010). "Cooking methods and storage treatments of potato: Effects on carotenoids, antioxidant activity, and phenolics." AM J POTATO RES, 87(6), 479-491.

[3.] Damşa, F., Woinaroschy, A., Olteanu, G. (2014). Quantification of total flavonoid and total anthocyanin content from purple potato, REV CHIM BUCHAREST 66(2), 227-229

[4.] Dao, L. and Friedman, M. (1992). "Chlorogenic acid content of fresh and

processed potatoes determined by ultraviolet spectrophotometry." J AGRIC FOOD CHEM, 40(11), 2152-2156.

[5.] Dewanto, V., X. Wu, K. Adom and R. Liu (2002). "Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity." J AGRIC FOOD CHEM, 50(10), 3010-3014.

[6.] Ezekiel, R., N. Singh, S. Sharma and A. Kaur (2011). "Beneficial phytochemicals in potato - a review." FOOD RES INT, 50, 487-496.

[7.] FAOSTAT (2012). Food and Agriculture Organization of the United Nations, http://faostat3.fao.org (accessed November 2014).

[8.] Farris, S., Piergiovanni, L. (2009). Optimization of manufacture of almond paste cookies using response surface methodology. J FOOD PROCESS ENG, 32(1), 64–87.

[9.] Giusti, M.M., Jing, P. (2007). Analysis of anthocyanins. Food Colorants Chemical and Functional Properties, ISBN 978-0-8493-9357-0, 479-507.

[10.] Kim, K.S., Lee, S., Lee, Y.S., 2003. Anti-oxidant activities of the extracts from the herbs of Artemisia apiacea. J ETHNOPHARMACOL, 85(1), 69-72.

[11.] Kosieradzka, I., Borucki, W., Matysiak-Kata, I., Szopa, J., Sawosz, E., 2004. Transgenic potato tubers as a source of phenolic compounds. Localization of anthocyanins in the peridermis. J ANIM FEED SCI, 13(2), 87–92.

[12.] Morar, G., Cătană, C., Olteanu, Gh., Pamfil, D., Vâtcă, S., Oltean, M.I., Oroian, G., 2004. Morphophysiological aspects of some old valuable potato varieties from transilvania, regenerated at USAMV Cluj-Napoca. Bulletin USAMV Cluj, Seria Agriculture/Horticulture, ISSN: 1454-2382.

[13.] Mulinacci, N., Ieri, F., Giaccherini, C., Innocenti, M., Andrenelli, L., Canova, G. (2008). "Effect of Cooking on the Anthocyanins, Phenolic Acids, Glycoalkaloids, and Resistant Starch Content in Two Pigmented Cultivars of Solanum tuberosum L." J AGRIC FOOD CHEM, 56(24), 11830-11837.

[14.] Nara, K., Miyoshi, T., Honma, T., Koga, H. (2006). Antioxidative activity of bound-form phenolics in potato peel. BIOSCI BIOTECH BIOCH, 70(6), 1489–1491.

[15.] Navarre, D., R. Shakya, J. Holden and S. Kumar (2010). "The Effect of Different Cooking Methods on Phenolics and Vitamin C in Developmentally Young Potato Tubers." AM J POTATO RES, 87(4), 350-359.

[16.] Nicoli, M., M. Anese and M. Parpinel (1999). "Influence of processing on the antioxidant properties of fruit and vegetables." TRENDS FOOD SCI TECH, 10(3), 94-100.

[17.] Price, K., Bacon, J., Rhodes, M. (1997). "Effect of storage and domestic processing on the content and composition of flavonol glucosides in onion (Allium cepa)." J AGRIC FOOD CHEM, 45(3), 938-942.

[18.] Spanos, G., R. Wrolstad and D. Heatherbell (1990). "Influence of processing and storage on the phenolic composition of apple juice." J AGRIC FOOD CHEM, 38(7), 1572-1579.

[19.] Teow, C.C., Van-Den Truong, McFeeters, R.F., Thompson, R.L., Pecota, K.V., Yencho, G.C., 2007. Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colours. FOOD CHEM, 103(3), 829–838.

[20.] Wettasinghe, M., Shahidi, F. (1999). Evening primrose meal: A source of natural antioxidants and scavenger of hydrogen peroxide and oxygen-derived free radicals. J AGRIC FOOD CHEM, 47(5), 1801–1812.

[21.] Zhishen J., Mengcheng T., Jianming W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. FOOD CHEM, 64, 555-559.