BETALAINS, TOTAL POLYPHENOLS, AND ANTIOXIDANT CONTENTS IN RED BEETROOT PEEL (*CYLINDRA TYPE*)

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Abstract

Since foods go to waste during processing, investigation of how to improve the valuable products of extraction from the wastes is deniably effective way to save the planet. Beetroot is a chief source of natural betalain color compounds and phenolic compounds which are rich in antioxidant. The major attention of this project is to optimize process variables which are extraction time (10-60 minutes), operating temperature (20-50^{o}C), and ethanol solvent concentration (25-75%) for effective extraction of valuable compounds such as betalains, total polyphenols, and antioxidant activity from beetroot peel. Spectrophotometric analysis was applied for quantification of those compounds. Process optimization was carried out using Design Expert (11.0.3) statistical software. Lowest solvent concentration (25 % v/v) together with highest temperature (50^{o}C) and extraction time (50 min) brought more amounts of outcomes. It can be noted that extraction process can be improved by controlling operating time and temperature avoiding unnecessary much usage of costly solvent.

Keywords: betalains, phenolic, antioxidant, ethanol, beetroot peel

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1. Introduction

Natural colorants such as carotenoids, anthocyanins, and betalains are noble health promoting substituents of artificial dyes. Predominant sources of nitrogen containing betalain compounds are red colored vegetables such as optunia, dragon fruit, swiss chard, pitaya and red beetroot (AKBAR HUSSAIN et al., 2018). Beetroot is standing first in the list of interest by consumers regarded as a rich antioxidant vegetable and accompanied distinct biological properties. Apart from that, beetroot contains noticeable amount of betalain and phenolic compounds.

Betalain which is well known for its better stability at pH (between 3 and 7) than anthocyanins (ANNA et al., 2006) can basically be divided into two main color components which are betacyanin and betaxanthin. Betacyanin is responsible for red-violet pigment named betanin and chiefly used in food industries. Betanin containing phenolic and cyclic amine groups strongly exhibits radical scavenging properties (ANNA et al., 2006). Unlike betacyanin, application of yellow betaxanthin as colorant is limited by its sensitivity (CAI et al., 2005). According to KATHIRAVAN et al. (2015), betacyanin is more tolerant to reduce color than betaxanthin during storage.

Solvents are basically used to complete the extraction process of betalain and phenolic compounds. Water, methanol-water, and ethanol-water are commonly used solvents in different ratio. Since betalains are sensitive to process conditions, extraction of betalains can be upgraded by adjusting pH (acidification) and temperature. Fermentation or enzymation can reduce free sugar content thereby consequently raising the quantity of betalains (DELGADO-VARGAS et al., 2010). CZAPSKI et al. (2009) explored that the correlation between red betacyanin and antioxidant is considerable whereas yellow betaxanthin shows indescribable correlation. Phenolic compounds are predominantly found in all edible plants in different forms (conjugated ring structure and hydroxyl groups) possessing strong free radicals and ROS scavenging power (MANACH et al., 2004).

Few reports for correlation between betaxanthin and betacyanin, TPC and antioxidant extracted from beetroot peel are available so far. Besides, process variables to improve the extraction of these compounds have hardly been considered in previous reports. The role of solvent in maximizing recovery of these valuable compounds from wastes is the major attention of this experiment.

2. Materials and Methods

2.1. Juice Extraction

Beetroot peel extracts were prepared as follows: fresh beetroots (Cylindra type) were processed right away after harvesting. Cleaned beetroots were peeled manually. Afterwards, the peels were grinded by pulverizer to get the pulp. Pulp (15) g was weighed and mixed with 150 mL of aqueous ethanol solvent (25, 50, 75 % v/v ethanol). Single stage batch extraction was carried out for (10, 35, 60 minutes) at (20, 35, 50^{o}C) by laboratory extractor before obtaining clear supernatant from centrifugation at 6000 rpm for 25 minutes.

2.2. Spectrophotometric Analysis

Color compounds, total polyphenol content, and antioxidant assay were performed by spectrophotometer (Genesys 5 UV-visible). Samples were diluted in appropriate ratios with distilled water for each analysis. For color compound detections, the absorbances were recorded at 480 nm for betaxanthin compound and 535 nm for betacyanin compound. Concentrations of the respective betalain compounds were calculated as follow;

$$BC = \frac{A\ x\ MW\ x\ DF\ x\ 1000}{\varepsilon\ x\ L}\ (mg/L)$$

where *A* is the absorbance; *MW* is the molecular weight; *DF* is the dilution factor; $\mathcal{E}$ is the molar extinction coefficient and *L* is the path length. Molecular weight and molar extinction coefficient for betaxanthin are (*MW*= 308 g/mol and $\mathcal{E}$= 48,000 L/(mol · cm) in water), and for betacyanin (*MW*= 550 g/mol and $\mathcal{E}$= 60,000 L/(mol · cm) in water) (PANDEY et al., 2018; RAVICHANDRAN et al., 2013). Analysis for TPC contents of each sample were performed by Folin-Ciocalteu method. Quantification of TPC was done at 760 nm and calculated as follow:

$$TPC = \frac{A\ x\ 2500\ x\ DF}{S\ x\ a}\left[\frac{mg\ GAE}{L}\right]$$

whereby: *A* – measured absorbance; *DF* – dilution factor; *S* – amount of sample; *a* – slope of calibration curve; *GAE* – gallic acid was used as a standard for calibration (BUENO et al., 2012). Antioxidant capacity was determined by Ferric reducing antioxidant power (FRAP) method as described by BENZIE & DEVAKI. (2018) with some modification and the absorbance was recorded at 593 nm. Ascorbic acid (*ASE*) was applied as a standard for calibration and express as ascorbic acid equivalent. The calculation was done using the equation;

$$AC = \frac{A\ x\ 1550\ x\ DF}{S\ x\ a}\left[\frac{mg\ ASE}{L}\right]$$

Whereas *A* – absorbance; *DF* – dilution factor; *S* – amount of sample; *a* – slope of calibration curve. The measurements of both TPC and Antioxidant were triplicated under the same conditions.

3. Results and discussions

Relationship between response and independent variables was investigated by choosing central composite design (CCD) of Design Expert Software version 11.0.3 for response surface methodology (RSM) performance. The response is well modeled by a quadratic function of independent variables so that the approximating function is the second-order model:

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i\ + \sum_{i=1}^{k} \beta_{ii} x_{ii}^2\ + \sum\sum_{i<j} \beta_{ij} x_i x_j + \epsilon$$

Table (1) shows ANOVA for quadratic model with square root transform. As can be seen in the table, lack of fit test is not significant for each response. *R*-squared values are not satisfactory for all response, however, the model can be regarded as significant from overall point of view considering *F*-test, standard deviation, mean, and coefficient of variation. *P*-value is lower than 0.05 in extraction time for all response revealing its significant effects to all outputs. Temperature is significant for total phenolic compound (TPC) and antioxidant whereas solvent concentration is significant for betalains.

Table (1) Analysis of Variance for the second-order quadratic function

Source	DF	Estimated coefficient				F Value			
		Betaxanthin	Betacyanin	TPC	Antioxidant	Betaxanthin	Betacyanin	TPC	Antioxidant
Model	4	9.11	12.07	16.23	24.30	6.04*	4.56*	5.69*	4.55*
A	1	0.71	0.95	1.41	1.92	10.37*	9.78*	6.10*	7.96*
B	1	0.18	0.01	2.09	1.45	0.67	0.002	13.44*	4.52*
C	1	-0.49	-0.57	0.00	-0.32	5.02*	3.54	0.00	0.22
A^2	1	-0.89	-0.95	-	-2.26	8.09*	4.91*	-	5.49*
C^2	1	-	-	-1.44	-	-	-	3.22	-
Residual	15	7.27	13.78	48.56	69.47				
Lack of Fit	10	3.75	8.48	23.73	22.39				
Pure Error	5	3.51	5.30	24.84	47.08				
Cor Total	19	18.96	30.52	122.25	153.76				
Std. Dev.		0.70	0.96	1.80	2.15				
Mean		8.67	11.60	15.51	23.17				
C.V. %		8.03	8.26	11.60	9.29				
R-Squared		0.62	0.55	0.60	0.55				

* Significant at "Prob > F" less than 0.05

The model operated the following regression equations for each dependent variable:

$$Sqrt\ (betaxanthin) = +6.951 + 0.127 * A + 0.012 * B - 0.019 * C - 0.001 * A^2$$

$$Sqrt\ (betacyanin) = +9.997 + 0.144 * A + 0.001 * B - 0.023 * C - 0.002 * A^2$$

$$Sqrt\ (TPC) = +3.616 + 0.056 * A + 0.139 * B + 0.231 * C - 0.002 * C^2$$

$$Sqrt\ (Antioxidant) = +14.447 + 0.329 * A + 0.097 * B - 0.013 * C - 0.004 * A^2$$

Whereas *A* means extraction time, *B* means temperature, and *C* represents solution concentration. According to model's suggestion, twenty experimental run were carried out with three different process variables: time (10 ~ 60 min), temperature (20 ~ 50 ºC), and aqueous ethanol solvent (25 ~ 75 % v/v) respectively, which were depicted in Table (2) accompanying their corresponding responses. As we can see in the table, the minimum and maximum yields of respective compounds were as follow: 46.29 ~ 112.68 mg l^{-1} (betaxanthin), 84.46 ~ 194.33 mg l^{-1} (betacyanin), 100.06 ~ 334.46 mg l^{-1} (TPC), and 267.37 ~ 866.81 mg l^{-1} (antioxidant).

3.1. Correlational effects of time and temperature

Contour diagrams (Figures 1 (a), (d), (g), and (j)) show the interaction effects of process time and temperature on betaxanthin, betacyanin, TPC, and antioxidant capacity whilst ethanol solvent (%) was fixed at the center point (50 % v/v). As model suggested that experimental parameter of time is significant for all responses, the higher yields of betalains, TPC, and antioxidant capacity were observed with enhancing processing time (from 30 min to 60 min) which is matched with WINITSORN et al. (2008) as the authors declared that concentration of solute in solvent phase depends only on extraction time. This is because the enough time to reach mass and heat transfer equilibrium is required in solid-liquid extraction (SATTLER & FEINDT. 1995). Betalains and antioxidant improved a bit with extended operating time, however, TPC content had increased dramatically. Beside the extraction time, TPC was also found to be affected by temperature. Likewise, a slight improvement in antioxidant capacity was observed with increasing temperature and it is in accordance with observation of PEDRENO & ESCRIBANO. (2001). Regarding temperature, there is no extinct changes in the amount of betalains. As for

TPC, both time and temperature had positive effects on the yield giving the highest TPC content at maximum operating time (60 min) and temperature (50 ºC). The possible explanation is that combination of longer extraction time and higher temperature might have softened the plant tissues accelerating the shift of phenolic compounds to the extraction medium (KUSHWAHA et al., 2018).

Table (2) Experimental scheme and results

	Process Variable			Response			
Run	Time (min)	Temperature (ºC)	Solvent (%)	Betaxanthin (mg l^{-1})	Betacyanin (mg l^{-1})	TPC (mg l^{-1})	Antioxidant (mg l^{-1})
1	10	20	75	46.29	85.31	104.28	347.94
2	10	20	25	46.46	84.46	100.06	267.37
3	35	35	50	63.91	115.62	267.76	488.34
4	60	50	75	68.62	122.22	325.07	548.41
5	35	20	50	90.52	165.24	304.40	567.45
6	35	35	50	112.68	194.33	260.71	866.81
7	35	35	50	83.93	145.81	266.82	492.74
8	10	50	75	60.66	110.00	279.03	481.02
9	60	20	75	76.23	148.13	193.54	498.11
10	35	35	75	59.97	109.39	228.30	607.50
11	60	20	25	85.73	163.41	189.78	506.90
12	60	35	50	75.97	141.29	330.70	622.64
13	10	35	50	57.66	108.78	193.54	459.04
14	35	35	50	88.55	149.11	334.46	659.26
15	60	50	25	94.03	155.34	295.00	709.07
16	35	35	25	98.22	172.58	281.85	695.89
17	35	50	50	73.32	116.84	327.88	570.87
18	35	35	50	82.22	143.12	133.88	450.74
19	10	50	25	73.92	133.10	262.12	495.67
20	35	35	50	83.84	157.18	255.54	558.18

3.2. Correlational effects of temperature and ethanol solvent percent

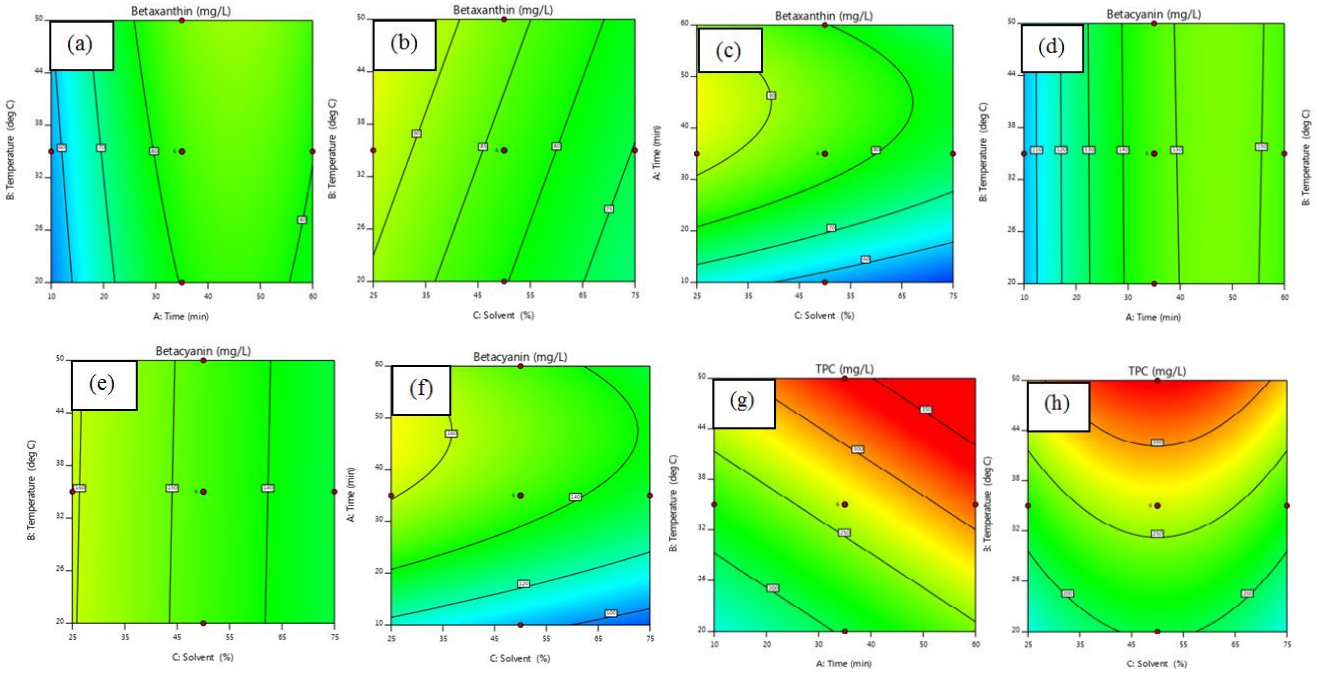
With regard to fixed extraction time (35 min), the interaction of temperature and ethanol solvent percent were probed for all responses (Figures 1 (b), (e), (h), and (k)). The contour plots proved that the effect of neither extraction temperature nor solvent on all outputs, except TPC, was found to be noteworthy. It means that the experimental parameters of these independent variables were not efficient to judge their effectiveness in accordance with dependent responses i.e, betalains and antioxidant. But, here again for TPC content, the combined effect of operating temperature and solvent was remarkable where the improvement of TPC content was found to be correlated with higher operating temperature and solvent concentration (Fig. 1 (h)). Over 3000 mg l^{-1} of phenolic compounds was obtained when temperature was increased up to 45 ºC and solvent concentration around 50 % v/v. This might have been due to possible earlier explanation discussed in the section of time and temperature interaction. Additionally, the solubility of the solute to be dissolved in the solvent is correlated with the temperature of the solvent (SATTLER & FEINDT, 1995). Although the calculated *p*-value from the model is greater than 0.05 in the case of solvent concentration, the interesting point here is that there is a decline in TPC amount as the concentration of aqueous ethanol was higher up to 50%. It could be concluded that extraction process reached the highest limit so that no more solvent is necessary to draw the compounds out

of the plant tissues. The concentration gradient which affects the diffusivity becomes greater with increasing solvent concentration and stops when the equilibrium is reached (CACACE & MAZZA, 2003).

3.3. Correlational effects of time and solvent concentration

Figures (c), (f), (i), and (l) exhibit the combined effect of time and solvent concentration on each response. The contour plots for betaxanthin and betacyanin reveal that higher operation time (over 45 min) accompanied with lower concentration of solvent (< 35 % w/v) provided the better yield of the pigments (Figs. 1 (c) and (f)). It is accordance in with observation of (KUSHWAHA et al., 2018) who claimed that higher yield of betalain pigments was achieved with low concentration of solvent. It means that lower concentration of solvent requires greater time and temperature due to the process needs to be driven to accomplish transfer of plant materials to extract medium (SANCHEZ-GONZALE et al., 2013). Therefore, the efficiency of extraction can be improved by raising process time and temperature with minimizing the solvent concentration. Notably, TPC content became greater with time when temperature was fixed at 35 ºC though solvent concentration affected the yield to some extent which is in contrast condition with the observation of PANDEY et al. (2018) who pointed out that no benefit in yield was resulted with increasing time. The possible reason here is that the different genotype of raw materials can vary the effectiveness of process variables on the responses (SAWICKI et al., 2016). As shown in Fig. 1 (l), it is obvious that antioxidant was independent on aqueous ethanol concentration but a bit increased in quantity was found in higher extraction time. The possible explanation might be the same with betalains since the model estimated that betalain is correlated with antioxidant in positive term i.e, 0.8.

Fig. 1 Contour plots for betalains, total phenolic, and antioxidant contents in beetroot peel extracts



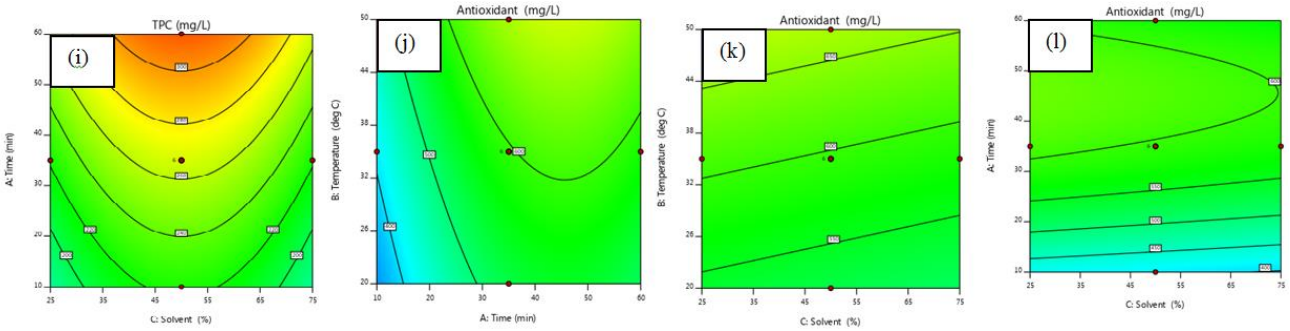
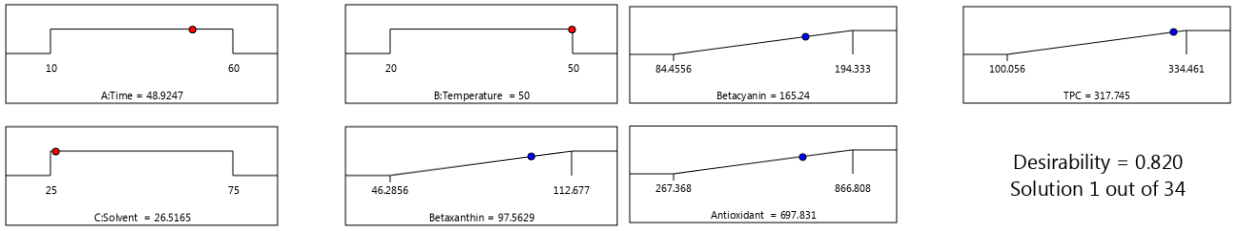


Fig. 2 Optimum condition of process variables for corresponding responses



4. Conclusion

The optimum condition of process variables under estimation was chosen by the model depending on the overall highest yield of betaxanthin (97.56 mg l^{-1}), betacyanin (165.24 mg l^{-1}), phenolic compounds (317.75 mg l^{-1}), and antiradical activity (697.83 mg l^{-1}) with desirability of 0.82 (Fig. 2). In our experiment, we mainly paid attention on interactions of time, temperature, and solvent concentration in order to supply the improvement of valuable compounds extraction. To sum up, the application of central composite design in approaching to highest outcome of can be effective in extending these compounds applications in different fields with minimal processing costs.

References

1. AKBAR HUSSAIN E., SADIQ Z., ZIA-Ul-HAQ, M. (2018) Sources of Betalains. In: Betalains: Biomolecular Aspects. Springer, Cham, 15-32.
2. ANNA, G. S., HENRYK. S., PAULINA, M. (2006) Betanin, the main pigment of red beet-molecular origin of its expectionally high free radical scavenging activity. *Food Addit Contam*. 23 (11): 1079-1087.
3. BENZIE, I. F. F., DEVAKI, M. (2018) Measurement of Antioxidant Activity & Capacity: Recent Trends of Applications, First Edition. John Wiley & Sons Publishers, 77-106.
4. BUENO, F. G., MACHARETH, M. A. D., PANIZZON, G. P., LOPES, G. C., MELLO, J. C. P., LEITE-MELLO, E. V. S. (2012) Development of a uv/vis spectrophotometric method for analysis of total polyphenols from *Caesalpinia peltophoroides* benth. *Quim. Nova*, 35(4): 822-826.
5. CACACE, J. E., MAZZA, G. (2003) Mass transfer process during extraction of phenolic compounds from milled berries. *J. Food Eng.* 59(4): 379–389.
6. CAI, Y. Z., SUN, M., CORKE, H. (2005) Characterization and application of betalain

pigments from plants of the Amaranthaceae. *Trends Food Sci. Technol.* 16(9): 370–376.
7. CZAPSKI, J., MIKOŁAJCZYK, K., KACZMAREK, M. (2009) Relationship between antioxidant capacity of red beet juice and contents of its betalain pigments. *Pol. J. Food Nutr. Sci*. 59 (2): 119-122.
8. DELGADO-VARGAS, F., JIMENEZ, A. R., PAREDES-LOPEZ, O. (2000) Critical reviews in food science and nutrition. Natural pigments: Carotenoids, anthocyanins, and betalains — characteristics, biosynthesis, processing, and stability. *Critical Reviews in Food Science and Nutrition*, 40(3):173-289.
9. KATHIRAVAN, T., NADANASABAPATHI, S., KUMAR, R. (2015) Pigments and antioxidant activity of optimized Ready-to-Drink (RTD) Beetroot (Beta vulgaris L .) - passion fruit (Passiflora edulis var . flavicarpa) juice blend. *Croat. J. Food Sci. Technol.* 7(1): 9-21.
10. KUSHWAHA, R., KUMAR, V., VYAS, G., KAUR, J. (2018) Optimization of different variable for eco-friendly extraction of betalains and phytochemicals from beetroot pomace. *Waste Biomass Valor*. 9: 1485-1494.
11. MANACH, C., SCALBERT, A., MORAND, C., RÉMÉSY, C., JIMÉNEZ, L. (2004) Polyphenols : food sources and bioavailability . *Am J Clin Nutr*. 79: 727-747.
12. PANDEY, G., PANDEY, V., PANDEY, P. R., THOMAS, G. (2018) Effect of extraction solvent temperature on betalain content, phenolic content, antioxidant activity and stability of beetroot (*Beta Vulgaris L.*) power under different storage conditions. *Plant Archives.* 18(2): 1623-1627.
13. PEDRENO, M. A., ESCRIBANO, J. (2001) Correlation between antiradical activity and stability of betanine from *Beta vulgaris L* roots under different pH, temperature and light conditions. *J. Sci. Food Agric*. 81: 627–631.
14. RAVICHANDRAN, K., SAW, N. M. M. T., MOHDALY, A. A. A., GABR, A. M. M., KASTELL, A., RIEDEL, H., CAI, Z., KNORR, D., SMETANSKA, I. (2013) Impact of processing of red beet on betalain content and antioxidant activity. *Food Res. Int.* 50(2): 670-675.
15. SANCHEZ-GONZALEZ, N., JAIME-FONSECA, M. R., MARTIN-MARTINEZ, E. S., ZEPEDA, L. G. (2013) Extraction, stability, and separation of betalains from opuntia joconostle cv. using response surface methodology. *J. Agric. Food Chem.* 61(49): 11995-12004.
16. SATTLER, K., FEINDT, H. J. (1995) Thermal Separation Process: Principles and Design. Prof. H. Weinheim, CCNY, 393-470.
17. SAWICKI, T., BĄCZEK, N., WICZKOWSKI, W. (2016) Betalain profile, content and antioxidant capacity of red beetroot dependent on the genotype and root part. *J. Funct. Foods.* 27: 249-261.
18. WINITSORN, A., DOUGLAS, P. L., DOUGLAS, S., PONGAMPAI, S., TEPPAITOON, W. (2008) Modeling the extraction of valuable substances from natural plants using solid – liquid extraction. *Chem. Eng. Commun.* 195 (11): 1457-1464.