OPTIMISATION OF ORGANIC SOLVENT EXTRACTION OF TOTAL CAROTENOIDS FROM BULGARIAN TOMATO PROCESSING WASTE

M. Nikolova, Ph.D., Head Assistant Professor[1], *E-mail*: milena_nikolova86@abv.bg
T. Prokopov, Ph.D., Professor[1], *E-mail*: tsvetko_prokopov@abv.bg
D. Taneva, Ph.D., Associate Professor[1], *E-mail*: don_taneva@abv.bg
N. Dimitrov, Ph.D., Associate Professor[2], *E-mail*: bussy@mail.bg
[1]Department of Engineering Ecology
[2]Department of Technology of Cereal, Bread and Confectionery Products
University of Food Technologies, 26 Maritsa blvd., 4002 Plovdiv, Bulgaria

Abstract. The quantity of tomato wastes combined with beneficial characteristics of components of the wastes justifies the great interest of researchers and manufacturers in extracting of carotenoids from this low cost material. In this study the response surface (RSM) approach and Box-Behnken design (BBD) were used to explore the possibility of modelling and optimisation the organic solvent extraction of total carotenoids from Bulgarian tomato processing waste (TPW). Bulgarian TPW consists of tomato pomace was used in this study. Dried and ground TPW was plased in the extraction flask and stirred with acetone at various extraction conditions. The extract obtained was vacuum filtered through filter paper and was subjected to spectrophotometrically total carotenoids content determination. A three-variable, three-level BBD of RSM was employed in optimisation the extraction conditions for the highest recovery of total carotenoids from Bulgarian TPW. A response surface quadratic model was developed and statistical analysis was carried out. Data concerning model adequacy tests indicated that the values of R^2 and R_{adj}^2 for quadratic model were the higest in comparison to the other models. The obtained F-value of 38.65 implies the model was significant ($p < 0.05$) and could be used for optimisation. The effects of extraction temperature, solvent to solid ratio and extraction time were significant in total carotenoids yield. Total carotenoids content ranged from 9.78 to 25.28 mg/100 g dried TPW. The predicted values of total carotenoids content were closed to the experimental observed values. By use of RSM the optimal extraction conditions were determined as follow: extraction time of 90 min, solvent to solid ratio of 60 mL/g and extraction temperature of 50ºC. The results obtained showed that predicted (28.40 mg/100 g dried TPW) and experimental (28.86±0.92 mg/100 g dried TPW) values of total carotenoids content were not significant different ($p > 0.05$).

Key words: optimization, tomato**,** waste**,** carotenoids**,** extraction.

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Introduction. Formulation of the problem

Food processing wastes have long been considered as a mater of treatment, minimization and prevention due to the environmental effects induced by their disposal. Nowadays, food wastes accounts as a source of valuable nutraceuticals [1].

Tomatoes are considered extremely valuable vegetables in terms of food. They are consumed in all regions of the world either fresh, processed or preserved. Commercial processing of tomatoes produced a large amount of waste. According to FAO data, 65–85% of the fresh tomatoes produced are processed and this industrial treatment generates around 10–40% of waste products, consist mainly of seeds and skins [2-4].

The quantity of tomato wastes combined with beneficial characteristics of components of the wastes justifies the great interest of researchers and manufacturers in extracting of carotenoids from this low cost material [5].

Modelling and optimisation of the carotenoids extraction are essential for commercial application of the extraction process. The importance of the results from pesent research is the development of the model applicable for prediction and optimisation of the tomato carotenoids organic solvent extraction from Bulgarian tomato processing waste.

Analysis of recent research and publications

The conventional extraction of tomato carotenoids includes the use of common organic solvents and solvent mixtures [6]. Improved methods such as enzyme-assisted and ultrasound-assisted extractions have been applied nowadays [7-10].

Bulgarian tomato cultivars "Stella", "Topaz", "Aquarius F_1", "Jaqueline", "Marigold" and "Carobeta" are the mostly used in tomato processing industry and carotenoids profile of the peels of these cultivars was established in our previous study [11]. The effect of treatment parameters of conventional organic solvent extraction on carotenoids recovery from tomato peels of Bulgarian industrial varieties was also observed [12].

Box-Behnken design (BBD) in Response surface methodology (RSM) is a statistical tool used recently in modelling and optimisation of the carotenoids extraction from different plant materials. The combined effects of extraction temperature, solvent to solid ratio and extraction time were most often studied [13-16]. However, the information in the literature regarding optimisation of organic solvent extraction of carotenoids from Bulgarian TPW is missing.

Therefore, the purpouse of this study is to used the RSM approach and BBD to explore the possibility of modelling and optimisation the organic solvent extraction of total carotenoids from Bulgarian TPW.

To achieve this goal, the following **objectives** were performed:

– developing of three-variable, three-level BBD of RSM in optimisation of the extraction conditions for the highest recovery of total carotenoids from Bulgarian TPW;

– conducting the organic solvent extraction of tomato carotenoids for various process parameters;

– determination of the optimal parameters for the organic solvent extraction of total carotenoids from Bulgarian TPW;

– running the additional experiments for verification of optimized extraction conditions and predicted value of total carotenoids content.

Research materials and methods

TPW consists of commercial dried tomato pomace was obtained from “Bulcons” JSC (Parvomay, Bulgaria) factory. Immediately after receiving it was, ground in laboratory mill (Bosch MKM 6003, Germany), and sieved through a 1.0 mm sieve. The moisture content of the dry ground TPW was determined by gravimetric method at 105°C and was found to be 9.01±0.21%. The resultant material was kept in glass jars closed with aluminium foil at 4°C in dark conditions until analysis. Acetone of analytical grade was purchased from “Sigma” (Germany).

Extraction of carotenoids was performed into 250 mL conical glass flask wrapped with aluminium foil and plased in a temperature-controlled (±1°C) water bath. Dried and ground TPW (1.00 g) was plased in the extraction flask and stirred with acetone at various extraction conditions by magnetic stirrer (VELP Scientifica, Italy) at 400 rpm. The extract obtained was vacuum filtered through MN640de filter paper and was subjected to carotenoids content determination.

Total carotenoids content of the extracts was determined spectrophotometrically (UV-VIS Helios Omega, USA) at room temperature using 1-cm path length quartz cuvette and expressed as mg of extracted carotenoid per 100 g of TPW on dry basis, according to procedure described by Manuelyan [17].

Three-variable, three-level BBD of RSM was applied in optimisation the extraction conditions for the highest recovery of total carotenoids from Bulgarian TPW with 15 runs, including 3 replicates at the center point [15,18]. Independent variables, such as extraction time (X_1, min), solvent to solid ratio (X_2, mL/g) and extraction temperature (X_3, ºC) and their ranges were determined on the base of single factor experimental analysis reported in our previous study [12] and are shown in Table 1. Limiting factors for the choise of the upper levels of the independent variables were the boiling point of acetone, the need to avoid possible isomerisation and/or oxidation of carotenoids, the balance between high cost and solvent wastes and avoidance of insufficient mixing and saturation effects [12].

Table 1 – Independent variables and their codded and actual levels used in Box-Behnken design

Independent variables	Levels		
	-1	0	1
Extraction time (X_1, min)	10	50	90
Solvent to solid ratio (X_2, mL/g	10	35	60
Extraction temperature (X_3, ºC)	20	35	50

The experiments were run in triplicate and the average concentration of total carotenoids was taken as response function (Y, mg/100 g). Experimental data were fitted to the second-order regression model, as shown in following equation:

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii} X_i^2 + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij} X_i X_j , \quad (1)$$

where β_0, β_i, β_{ii} and β_{ij} are the intercept, regression coefficients of the linear, quadratic and interaction terms of the model, respectively, while X_i and X_j are the independent variables and Y is the dependent variable.

Verification of optimized extraction conditions and predicted value of total carotenoids content were done in triplicate by running additional experiments.

The experimental design and statistical analysis were performed using Design-Expert® Software, v11 (State-Ease, Inc., Minneapolis, MN, USA, www.statease.com). The model adequacy was checked by coefficient of determination (R^2) and adjusted coefficient of determination (R_{adj}^2). Analysis of variance (ANOVA), F-test and p-value at 5% level of significance were applied for determination of model significance.

Results of the research and their discussion

The influence of extraction time (X_1, min), solvent to solid ratio (X_2, mL/g) and extraction temperature (X_3, ºC) on the total carotenoids organic solvent extraction is presented in Table 2. Total carotenoids content ranged from 9.78 to 25.28 mg/100 g dried TPW. The predicted values of total carotenoids content determined by regression equation (2) were closed to the experimental observed values.

The sequential model sum of squires and model summary statistic tools were applied to check the model adequacy and the results are shown in Table 3. Data obtained for model adequacy indicated that the values of R^2 and R_{adj}^2 for quadratic model were the higest in comparison to the other models, excluding the cubic model which was aliased.

Table 4 shows the results of fitting quadratic model to the experimental data. The empirical regression equation represents the total carotenoids content (Y, mg/100 g dried TPW) as function of independent variables is given below:

$$Y = 15.5117 + 0.9975\,X_1 + 2.2763\,X_2 + 3.8463\,X_3 - 0.015\,X_1X_2 + 3.3X_1X_3 + \\ + 1.6475\,X_2X_3 + 2.1704\,X_1^2 - 0.0821\,X_2^2 - 1.2521\,X_3^2 \quad (2)$$

Table 2 – Coded Box-Behnken design with the observed and predicted values of total carotenoids content

Run	Coded variable levels			Total carotenoids content (mg/100 g)	
	X_1	X_2	X_3	Observed value[a]	Predicted value
1	1	-1	0	16.32	16.34
2	1	1	0	20.67	20.86
3	-1	1	0	18.91	18.89
4	0	0	0	15.06	15.51
5	1	0	1	25.28	24.57
6	-1	0	-1	14.18	14.89
7	0	1	1	21.43	21.95
8	1	0	-1	9.78	10.28
9	-1	0	1	16.48	15.98
10	0	-1	1	13.41	14.10
11	0	1	-1	11.65	10.96
12	0	0	0	15.44	15.51
13	0	0	0	16.04	15.51
14	-1	-1	0	14.50	14.31
15	0	-1	-1	10.22	9.70

[a]Mean of triplicate determinations

On the base of results from ANOVA the obtained F-value of 38.65 implies the model was significant ($p < 0.05$) and could be used for optimization. P-values less than 0.05 indicate model terms are significant. In this case linear terms (X_1, X_2 and X_3), interaction terms ($X_1.X_3$ and $X_2.X_3$) and quadratic-terms (X_1^2 and X_3^2) are significant model terms. However, the interaction ($X_1.X_2$) as well as the quadratic-term coefficient of solvent to solid ratio (X_2^2) were found to be insignificant ($p > 0.05$) as indicated in Table 4. The Lack of fit F-value of 4.14 implies the lack of fit was not significant ($p > 0.05$) relative to the pure error, which indicated that the model adequately fits the experimental data. Many researches also reported adequate fitting of the experimental data to second-order regression model [13-16].

Table 3 – Selection of satisfactory model for organic solvent extraction of total carotenoids from TPW

Sequential model sum of squires						
Source	**Sum of squares**	**Df**	**Mean square**	**F-value**	**p-value**	**Remark**
Mean vs Total	3819.71	1	3819.71			
Linear vs Mean	167.76	3	55.92	7.42	0.0055	
2FI vs Linear	54.42	3	18.14	5.09	0.0292	
Quadratic vs 2FI	24.95	3	8.32	11.71	0.0107	Suggested
Cubic vs Quadratic	3.06	3	1.02	4.14	0.2007	Aliased
Residual	0.4928	2	0.2464			
Total	4070.39	15	271.36			
Model summary statistics						
Source	**Std. dev.**	**R^2**	**R_{adj}^2**	**Predicted R^2**	**PRESS**	**Remark**
Linear	2.75	0.6692	0.5790	0.2959	176.51	
2FI	1.89	0.8863	0.8010	0.4126	147.26	
Quadratic	0.8429	0.9858	0.9603	0.8003	50.06	Suggested
Cubic	0.4964	0.9980	0.9862		*	Aliased

*Case(s) with leverage of 1.0000: PRESS statistic not defined. 2FI – two factor interaction

The results indicated that the extraction temperature was the most significant linear term variable affected the total carotenoids extraction from TPW, followed by interaction term of extraction time and extraction temperature and linear term if solvent to solid ratio. The quadratic term of extraction time also showed high significant positive effect on the studied response (Table 4).

Table 4 – ANOVA for response surface quadratic model for organic solvent extraction of total carotenoids from TPW

Source	Sum of squares	Df	Mean square	F-value	p-value	Remark
Model	247.13	9	27.46	38.65	0.0004	significant
X_1	7.96	1	7.96	11.20	0.0204	
X_2	41.45	1	41.45	58.34	0.0006	
X_3	118.35	1	118.35	166.57	<0.0001	
$X_1.X_2$	0.0009	1	0.0009	0.0013	0.9730	
$X_1.X_3$	43.56	1	43.56	61.31	0.0005	
$X_2.X_3$	10.86	1	10.86	15.28	0.0113	
X_1^2	17.39	1	17.39	24.48	0.0043	
X_2^2	0.0249	1	0.0249	0.0350	0.8589	
X_3^2	5.79	1	5.79	8.15	0.0356	
Residual	3.55	5	0.7105			
Lack of fit	3.06	3	1.02	4.14	0.2007	not significant
Pure error	0.4928	2	0.2464			
Cor Total	250.14	14				

The effect of extraction time (X_1) and solvent to solid ratio (X_2) at fixed temperature of 35ºC on total carotenoids content (Y, mg/100 g dried TPW) is plotted on Figure 1. The results indicated that liquid to solid ratio had strong positive linear effect on total carotenoids content and the extraction time had significant positive linear and quadratic effects. The increasing of extraction time above 50 min enhanced the total carotenoids content.

Figure 2 illustrated the effect of extraction time (X_1) and extraction temperature (X_3) at fixed solvent to solid ratio of 35 mL/g on total carotenoids content (Y, mg/100 g dried TPW). The analysis of the results indicated that the optimal time and temperature of the total carotenoids extraction were about 90 min and 50ºC. Kaur et al. (2008) also reported optimal temperature of 50ºC for extraction of lycopene from tomato processing waste skin [13].

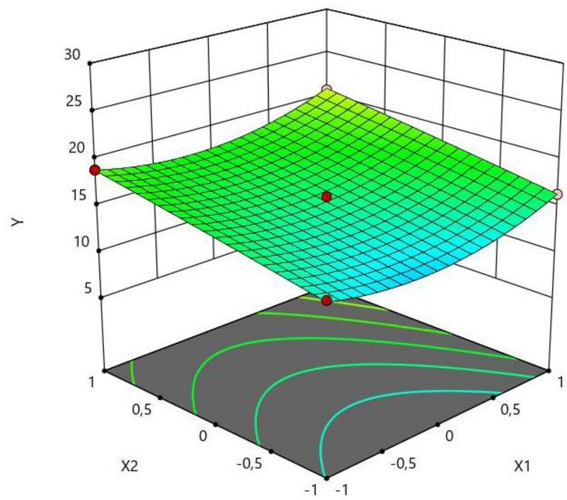

Fig. 1. Response surface plot of the effects of extraction time (X_1) and solvent to solid ratio (X_2) at temperature 35ºC in organic solvent extraction on total carotenoids content (Y, mg/100 g dried TPW)

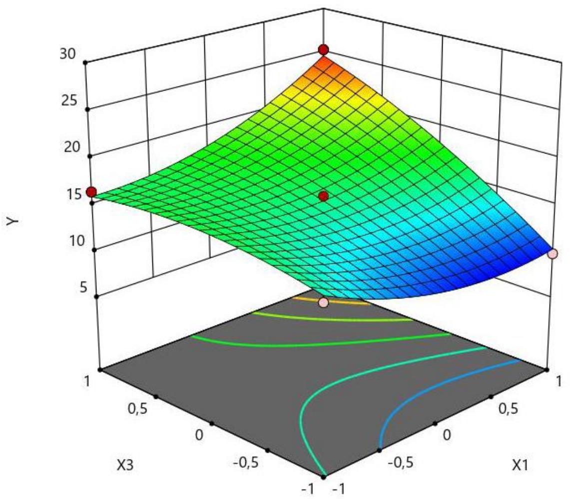

Fig. 2. Response surface plot of the effects of extraction time (X_1) and extraction temperature (X_3) at solvent to solid ratio 35 mL/g in organic solvent extraction on total carotenoids content (Y, mg/100 g dried TPW)

The effect of solvent to solid ratio (X_2) and extraction temperature (X_3) at fixed extraction time of 50 min on total carotenoids content (Y, mg/100 g dried TPW) is shown in Figure 3. The results indicated that the increasing of solvent to solid ratio and extraction temperature led to increasing total carotenoids content and the optimal solvent to solid ratio and temperature for maximum total carotenoids extraction were about 60 mL/g and 50ºC.

The results from our study indicated that the increases of temperature, liquid to solid ratio and time of extraction increases the amount of total carotenoids to be extracted by the solvent from TPW. Similar results has been reported in another research concerning organic solvent extraction of lycopene from TPW [10].

The optimized conditions obtained by RSM was used to validate the second-order regression model of extraction of total carotenoids from TPW. The results indicated that maximum total carotenoids content of 28.40 mg/100 g dried TPW could be achieved at extraction time of 90 min, solvent to solid ratio of 60 mL/g and extraction temperature of 50ºC. The literature reported carotenoid content of dry tomato by-products collected from a commercial tomato processing plants may vary, depending mostly on the tomato variety and on the industrial processing method and also on the solvent used and extraction conditions. For instance, total carotenoids (expressed as lycopene) extracted with aceton (at 50ºC, solvent to solid ratio of 10 mL/g and three sucsessive extractions for 30 min each) from tomato industrial waste (mixture of skins and seeds) was reported to be 51.9 mg/kg dry basis [5,6]. The difference compare to our result may be mainly due to the tomato variety features and method of industrial tomato processing.

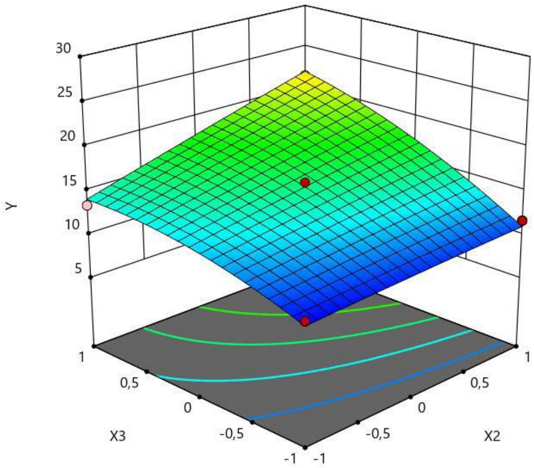

Fig. 3. Response surface plot of the effects of solvent to solid ratio (X_2) and extraction temperature (X_3) at extraction time 50 min in organic solvent extraction on total carotenoids content (Y, mg/100 g dried TPW)

For model verification and validation of optimal extraction conditions the additional experiments were conducted in triplicate under the optimized conditions and the average total carotenoids content was found to be 28.86±0.92 mg/100 g dried TPW which was very closed to the RSM predicted value.

Conclusions

1. Three-variable, three-level BBD in RSM was developed to determine the optimal extraction conditions in organic solvent extraction of total carotenoids content from TPW.

2. ANOVA indicated that the effects of extraction temperature, solvent to solid ratio and extraction time were significant in total carotenoids extraction. The second-order regression model was used in predicting the response function.

3. The optimal extraction conditions were determined as follow: extraction time of 90 min, solvent to solid ratio of 60 mL/g and extraction temperature of 50ºC.

4. The results obtained showed that predicted (28.40 mg/100 g dried TPW) and experimental (28.86±0.92 mg/100 g dried TPW) values of total carotenoids content were not significant different ($p > 0.05$). Therefore, the model obtained can be used for optimisation of total carotenoids organic solvent extraction from Bulgarian TPW.

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