Optimization of frozen wild blueberry vacuum drying process

Zdravko M. Šumić[1], Aleksandra N. Tepić[1], Stela D. Jokić[2], Radomir V. Malbaša[1]

[1]*University of Novi Sad, Faculty of Technology, Novi Sad, Serbia*
[2]*Faculty of Food Technology, Osijek, Croatia*

Abstract

The objective of this research was to optimize the vacuum drying of frozen blueberries in order to preserve health benefits of phytochemicals using response surface methodology. The drying was performed in a new design of vacuum dryer equipment. Investigated range of temperature was 46–74 °C and of pressure 38–464 mbar. Total solids, total phenolics, vitamin C, anthocyanin content and total color change were used as quality indicators of dried blueberries. Within the experimental range of studied variables, the optimum conditions of 60 °C and 100 mbar were established for vacuum drying of blueberries. Separate validation experiments were conducted at optimum conditions to verify predictions and adequacy of the second-order polynomial models. Under these optimal conditions, the predicted amount of total phenolics was 3.70 g CAE/100_{dw}, vitamin C 59.79 mg/$100g_{dw}$, anthocyanin content 2746.33 mg/100 g_{dw}, total solids 89.50% and total color change 88.83.

Keywords: blueberry, vacuum drying, response surface methodology, product quality.

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Blueberries (*Vaccinium myrtillus* L.) are a valuable fruit worldwide, due to their improved nutritive value and many health benefits. The use of blueberries as a medicine dates since the 16th century. It has been included in many pharmacopoeias, and used in medicine and pharmacy [1]. In addition, berry fruits and their extracts can nowadays be used as a component of functional foods, dietary foods or dietary supplements [2]. Blueberries are also known for their high anthocyanin and flavonoid content. Their high antioxidant capacity has been attributed to high anthocyanin pigment content; their health-promoting features are attributed to phenolic acids and flavonoids. Many scientific papers report protective effect of berries and berry extracts such as antioxidant [2–6], anti-inflammatory, hepatoprotective and anticarcinogenic [1,7]. Reported values can vary depending on the variety. Moreover, total phenolic, total anthocyanin content, and antioxidant activities were significantly higher in wild berries than in cultivated ones [8].

As there are no many data about wild blueberries, the aim of this research was to determine the main chemical composition, vitamin C, anthocyanin, phenolics of wild blueberry fruits from Kopaonik mountain region in Serbia. In the present study, we applied vacuum drying to produce dried blueberries. To preserve the main health benefits of phytochemicals of dried blueberries, the influence of temperature and pressure on the quality of the final blueberry dried product under the applied experimental range using response surface methodology (RSM) was investigated.

Correspondence: A. Tepić, University of Novi Sad, Faculty of Technology, Bul. cara Lazara 1, 21000 Novi Sad, Serbia.
E-mail: tepical@uns.ac.rs
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MATERIAL AND METHODS

Chemicals

Vitamin C, produced by J.T. Baker (Holland) was used as a standard. Standard substance and samples were dissolved/extracted in the solution of 3% *m*-phosphoric acid (Riedel-de Haën, Germany) in 8% acetic acid (J.T. Baker, Holland). Ammonia-acetate solution was used as a mobile phase (0.1 M; pH 5.1). Solutions were prepared in redistilled water with appropriate quality for HPLC analysis. Folin-Ciocalteu reagent was purchased from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Chlorogenic acid was purchased from Sigma (Sigma, St. Luis, MO, USA). All other chemicals and reagents were of analytical reagent grade.

Material

Fruits of wild blueberry were grown in the region of Kopaonik mountain, Serbia, and hand-harvested at commercial maturity stage in season 2011. The collected fruits were washed, frozen and stored at –35 °C until analysis.

Drying procedure

Drying was performed in a vacuum dryer prototype (Figure 1) constructed and installed at the Department of Food Preservation, Faculty of Technology, Novi Sad (Serbia). Experimental drying facility consists of a cylindrical vacuum chamber made of steel sheet with a volume of about 70 L. The vacuum pump provides pressure in the chamber of 2 mbar. The chamber is equip-

ped with the condensate collector. The aluminum tray is fixed in a special frame and connected to a balance.

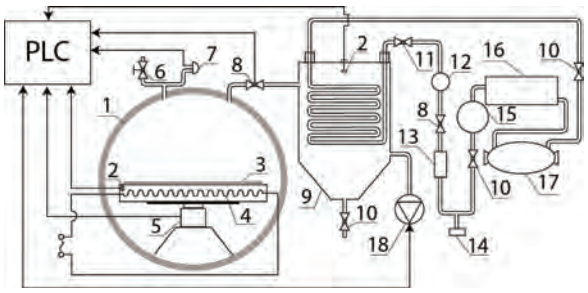

Figure 1. Schematic diagram of the vacuum drying equipment. 1: drying chamber; 2: temperature sensor; 3: sample holder; 4: heater; 5: load cell; 6: vacuum break-up valve; 7: pressure sensor; 8: solenoid valve; 9: condensate collector; 10: valves; 11: expansion valve; 12: flow indicator; 13: evaporator; 14: receiver; 15: pressure control; 16: condenser; 17: compressor; 18: vacuum pump.

The drying procedure control system (PLC) registers all working parameters (pressure in vacuum chamber, temperature on the heater surface and the change in product weight) during the drying process and the system controls the level of electric power supplied to the heaters to provide a product temperature not more than 75 °C.

The most relevant technical features relating for device are the following: 25–75 °C working temperature range; sensor sensitivity ±0.3 °C; 1.000 g balance maximum load; 0.03% balance sensitivity; 0.1 g balance resolution; 2–1000 mbar working pressure range, sensor sensitivity ±0.5.

The samples were uniformly arranged on the tray as a thin layer. Sample size was kept constant (about 400 g) for each experiment. Weight loss was recorded in 5 minute intervals and drying was continued until no mass change was detected (final moisture content in equilibrium). Drying runs were performed at pressures of 38 to 462 mbar and temperatures of 46 to 74 °C, according to the experimental plan given in Table 1. Drying times ranged from 5.5 to 29.1 h, depending on working conditions (temperature and pressure).

Table 1. The uncoded and coded levels of independent variables used in the RSM design

Independent variable	Symbol	Level				
		–1.414	–1	0	+1	1.414
Drying temperature, °C	X_1	46	50	60	70	74
Vacuum pressure, mbar	X_2	38	100	250	400	464

Experimental design

Response Surface Methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes. RSM is a critical technology in developing new processes and optimizing their performance. The objectives of quality improvement, including reduction of variability and improved process and product performance, can often be accomplished directly using RSM [9]. The central composite rotatable design (CCRD) was used for determining optimal drying temperature and vacuum pressure for drying process of frozen blueberries [10]. Drying air temperature (X_1) and vacuum pressure (X_2) were independent variables studied to optimize the drying process in terms of getting better final product quality (y). Investigated factors and levels tested were reported in Table 1. Experimental data were fitted with second order response surface model with the following form:

$$y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum \sum_{i<j} \beta_{ij} X_i X_j \quad (1)$$

where y are investigated responses (total solids, total phenolics, vitamin C, anthocyanin content and total colour change), β_0, β_j, β_{jj} and β_{ij} are constant coefficients of intercept, linear, quadratic, and interaction terms, respectively; X_i and X_j are coded independent variables (drying temperature and vacuum pressure).

Statistical analysis

Statistical analysis was performed using RSM software Design-Expert® v.7 (Stat-Ease, MN, USA). The results were statistically tested by the analysis of variance (ANOVA) at the significance level of p = 0.05. The adequacy of the model was evaluated by the coefficient of determination (R^2) and model p-value. A mathematical model was established to describe the influence of single process parameter and/or interaction of multiple parameters on each investigated response. Response surface plots were generated with the same software and drawn by using the function of two factors, and keeping the other constant.

Total solids

Total solids were determined by drying the samples at 105 °C until constant weight. Experiments were replicated three times for statistical purpose.

Total phenols

Dried blueberry samples were ground in a blender before the extraction. 10.0 g of this way prepared sample was transferred to volumetric flask and 50 ml of methanol, as extraction solvent, was added. Extraction was carried out for 24 h at the room temperature, after obtained extract was filtered. Prepared blueberry extracts were placed into a glass bottles and stored to prevent oxidative damage until analysis. The content of total phenolic compounds in blueberry extracts was

determined by Folin–Ciocalteu procedure [5,11] using chlorogenic acid as a standard. Absorbance was measured at 765 nm. Content of total phenolic compounds has been expressed as g of chlorogenic acid equivalent per 100 g of dried blueberries (g CAE/100 g_{dw}). Experiments were replicated three times for statistical purpose.

Vitamin C

2.5 g of ground dried blueberries was transferred to 25 ml volumetric flask, 3% m-phosphoric acid in 8% acetic acid was added and the mass was mixed for 5 min. The flask was filled up to the volume and filtered. Activated carbon was added to the filtered solution to remove the colour and filtered through filter paper (blue label) and membrane syringe filter with diameter pore of 0.45 μm. The filtrate was used for HPLC analysis of vitamin C at the HPLC system (Agilent 1100, USA) equipped with C-8 column and DAD detector. Mobile phase (0.1 M ammonia-acetate) flow rate was 0.4 ml/min and column temperature 37 °C. All analyses were performed in triplicate.

Anthocyanin content

Anthocyanin content (total monomeric anthocyanin content, TMA) in dried blueberries was determined as described by Giusti and Wrolstad [12], based on the pH-differential method previously described by Fuleki and Francis [13]. Blueberry extract absorbances were measured at 510 and 700 nm in 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5). Anthocyanin content was expressed as mg of cyanidine 3-glucoside equivalents per 100 g of dry weight (total solids) of blueberries. Experiments were replicated three times for statistical purpose.

Surface colour

The CIE *L*a*b** colour coordinates were measured using MINOLTA Chroma Meter CR-400 (Minolta Co., Ltd., Osaka, Japan). The apparent (surface) colour of samples was measured in terms of *L* (degree of darkness), *a* (degree of redness and greenness) and *b* (degree of yellowness and blueness). Finally, the total color change between blank white (L_0^*, a_0^* and b_0^*) and dried blueberry samples (L^*, a^* and b^*) was determined according to:

$$\Delta E = \sqrt{\left[\left(L_0 - L\right)^2 + \left(a_0 - a\right)^2 + \left(b_0 - b\right)^2 \right]} \quad (2)$$

Samples were placed on the measure head of Chroma Meter and measurements of color were performed for all prepared samples. A standard white color was used for calibration. Experiments were replicated five times for statistical purpose.

RESULTS AND DISCUSSION

Response surface methodology (RSM) was used in order to optimize vacuum drying process of blueberries. In this study, effects of drying temperature (46–74 °C) and pressure (38–462 mbar) on the total solids, total phenolics content, vitamin C, antioxidant activity, total anthocyanin content and total colour change of the blueberries dried in vacuum drier were investigated (Table 2). Table 3 shows the corresponding *p*-values for selected response variables of blueberries for each obtained coefficients and interactions. The effect of the linear, quadratic or interaction coefficients on the response was tested for significance by analysis of variance (ANOVA). Analysis of variance (Table 4) shows that the regression models for all investigated responses were statistically relevant with a significance

Table 2. The experimental design and data for the response surface analysis

Run	Temp. °C	Vacuum pressure mbar	Total solids %	Total phenolics content g CAE/100 g_{dw}	Vitamin C mg/100 g_{dw}	Anthocyanin content mg/100 g_{dw}	Total color change
1	64	250	89.11	3.6395	52.622	2455.10	89.89
2	60	400	87.98	2.9025	47.699	1986.40	89.68
3	60	100	90.26	3.5061	67.593	2753.45	88.33
4	50	462	76.41	2.1529	36.760	1984.02	90.21
5	50	250	79.41	2.9782	36.991	2067.01	91.05
6	50	250	81.53	2.9891	37.649	1975.59	91.21
7	50	250	82.68	2.8828	39.859	1927.40	90.88
8	50	250	83.82	3.1804	36.814	1955.22	91.24
9	50	250	74.84	2.7565	34.482	1920.42	90.63
10	50	38	83.84	3.3039	35.699	2455.74	89.58
11	40	400	80.45	2.8211	32.703	1828.70	91.88
12	40	100	76.99	2.5993	28.154	1938.93	91.57
13	36	250	76.86	2.7003	34.805	1771.99	90.60

Table 3. Corresponding p-values for selected response variable of dried blueberries for each obtained coefficients; X_1: drying temperature; X_2: vacuum pressure; $p < 0.01$ highly significant; $0.01 \leq p < 0.05$ significant; $p \geq 0.05$ not significant

Response	Term				
	X_1	X_2	X_1^2	X_2^2	X_1X_2
Total solids	0.0009	0.3774	0.1533	0.6419	0.2777
Total phenolics	0.0058	0.0114	0.2141	0.1987	0.0889
Vitamin C	0.0010	0.3790	0.0664	0.7882	0.0515
Anthocyanin content	< 0.0001	< 0.0001	0.0234	0.0011	0.0007
Total color change	0.0096	0.1834	0.2439	0.1013	0.4465

Table 4. Analysis of variance (ANOVA) of the modelled responses; the recovery

Source	Sum of squares	Degree of freedom	Mean square	*F*-value	*p*-value
		Total solids			
Model	213.37	5	42.67	7.18	0.0111
Residual	41.63	7	5.95	–	–
Lack of fit	27.74	3	9.25	2.66	0.1838
Pure error	13.89	4	3.47	–	–
Total	255.00	12	–	–	–
		$R^2 = 0.9217$			
		Total phenolics			
Model	1.54	5	0.31	7.06	0.0117
Residual	0.31	7	0.044	–	–
Lack of fit	0.21	3	0.070	2.87	0.1677
Pure error	0.097	4	0.024	–	–
Total	1.85	12	–	–	–
		$R^2 = 0.8344$			
		Vitamin C			
Model	1094.23	5	218.85	8.05	0.0081
Residual	190.23	7	27.18	–	–
Lack of fit	175.39	3	58.46	15.75	0.0111
Pure error	14.84	4	58.46	–	–
Total	1284.46	12	–	–	–
		$R^2 = 0.8519$			
		Anthocyanin content			
Model	984000	5	196800	60.88	< 0.0001
Residual	22630.64	7	3232.95	–	–
Lack of fit	8700.87	3	2900.29	0.83	0.5415
Pure error	13929.77	4	3482.44	–	–
Total	1007000	12	–	–	–
		$R^2 = 0.9775$			
		Total color change			
Model	8.29	5	1.66	3.99	0.0495
Residual	2.91	7	0.42	–	–
Lack of fit	2.65	3	0.88	13.85	0.0140
Pure error	0.26	4	0.064	–	–
Total	11.20	12	–	–	–
		$R^2 = 0.8368$			

level ranging from $p < 0.0001$ (for anthocyanin content) to $p = 0.0495$ (for total colour change). The fitted model represent the experimental data well with high correlation coefficients, R^2, varying from 0.8344 to 0.9775, depending on investigated responses. The second order polynomial models used to express the investigated responses (y) as a function of independent variables (in terms of coded values) are shown in Table 5.

Total phenolic content of dried blueberries

Blueberries are a very valuable fruit due to their high concentration of phenolics and anthocyanins [6]. Total phenolic content in different varieties of blueberry can be from 251 [8], 300 to 384 [14], but can range up to 929 mgGAE/100 g [15]. The data about individual phenolics are different in the literature. You and others [14] identified seven phenolics in blueberries: caffeic acid, chlorogenic acid, *p*-coumaric acid, 4-*O*-feruloulquinic acid, 5-*O*-feruloylquinic acid, trans--ferulic acid and quercetin, with chlorogenic acid being the dominant. They did not found gallic acid, or catechin, which were dominant in the research of Sellapan and others [15]. Generally, depending on the cultivar, growing season and location, the content and profile of phenolic compounds vary [8].

In this study, the total phenolic content of dried blueberries varied from 2.15 to 3.64 g CAE/100 g_{dw} according to different investigated parameter levels. Total phenolic content was significantly influenced by linear term of drying temperature and pressure (Table 3). Furthermore, the interaction between temperature and pressure (X_1X_2) didn't have a significant effect on total phenolic content ($p = 0.0889$) as well as quadratic term of temperature ($p = 0.2141$) and quadratic term of pressure ($p = 0.1987$). The second order polynomial model used to express the total phenolic content (y_3) as a function of independent variables (in terms of coded values) are shown in Table 5. Figure 2 shows that total phenolic content in dried blueberries increased with increasing of drying temperature. It can be seen also that the total phenolic content increased slightly with increasing pressure until 260 mbar, while further increase did not show any significant change on total phenolic content.

Table 5. The second order polynomial models used to express the investigated responses (y) as a function of independent variables (in terms of coded values); X_1: drying temperature; X_2: vacuum pressure

Response	Second order polynomial model	Eq.
Total solids	$y_1 = 81.46 + 4.77X_1 - 0.81X_2 + 1.48X_1^2 - 0.45X_2^2 - 1.43X_1X_2$	(3)
Total phenolics	$y_2 = 2.96 + 0.29X_1 - 0.25X_2 + 0.11X_1^2 - 0.11X_2^2 - 0.21X_1X_2$	(4)
Vitamin C	$y_3 = 37.16 + 9.95X_1 - 1.73X_2 + 4.29X_1^2 + 0.55X_2^2 - 6.11X_1X_2$	(5)
Anthocyanin content	$y_4 = 1969.13 + 242.29X_1 - 193.05X_2 + 62.25X_1^2 + 115.4X_2^2 - 164.20X_1X_2$	(6)
Total color change	$y_5 = 91.00 - 0.81X_1 + 0.34X_2 - 0.31X_1^2 - 0.46X_2^2 + 0.26X_1X_2$	(7)

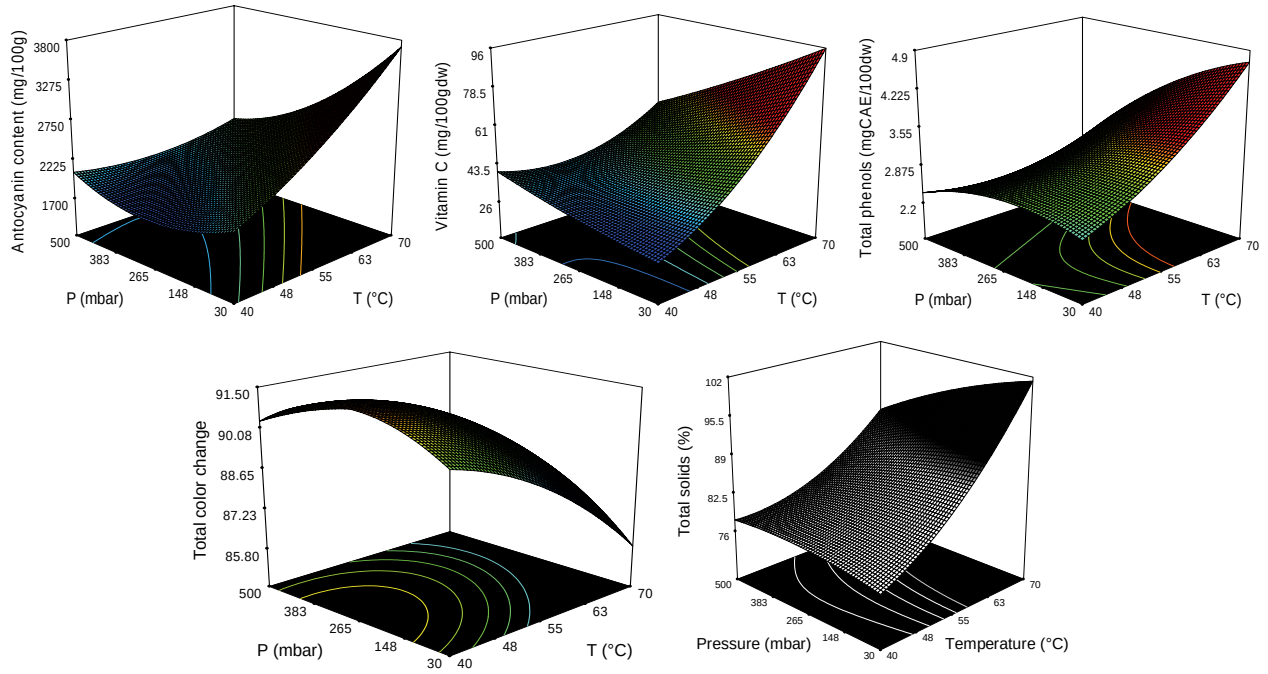

Figure 2. Surface plot for obtained responses as a function of vacuum pressure and drying temperature.

Anthocyanin content of dried blueberries

Besides their role as colour substances, anthocyanins are group of compounds which can provide many health benefits to humans. In general, total phenolics and total anthocyanins are higher in wild in comparison to cultivated fruits [8]. Sellapan and others [15] reported that blueberries had 87.4–197 mg anthocyanins per 100 g of fruit. Results obtained by You and others [14] showed that total anthocyanin contents in different cultivars was in the range from 116 to 224 mg/100 g, and there was even no significant difference between samples grown in organic and conventional conditions. Five main groups of anthocyanidins were identified in blueberries: cyanidin, delphinidin, peonidin, malvidin and petunidin [14,16]. They concluded that HPLC–MS method for individual anthocyanins and spectrophotometric method for total anthocyanins were reliable.

In this study, anthocyanin content was in the range from 1771.99 to 2753.45 mg/100 g_{dw}. According to the data from the Table 3, it can be seen that linear term of drying temperature had statistically significant influence on anthocyanin content of dried blueberries (p < < 0.0001). Furthermore, the quadratic terms of vacuum pressure and drying temperature also show significant effect on anthocyanin content of dried blueberries as well as the interaction between these two parameters (p = 0.0007). Effects of drying temperature and vacuum pressure on anthocyanin content of dried blueberries can be described by equation (Eq. (7)) and is presented in Figure 2. From this figure it can be seen that by increasing of drying temperature anthocyanin content significantly increase. With the increase of pressure to about 300 mbar, anthocyanin content slightly decreases. Further increase of pressure led to increase of antocyanin content in dried blueberries.

Vitamin C

Vitamin C is a compound that has many important biological functions in human body. When speaking about drying of fruit, the vitamin C content can serve as an indicator of severity of the drying process. In fresh blueberries, vitamin C content can range from 1.3 to 16.4 mg/100 g [6,17]. Also, depending on the cultivar, there can be a significant variation in the vitamin C content.

In this study, the vitamin C content of dried blueberries varied from 28.15 to 67.59 mg/100 g_{dw}. It is evident from Table 3 that linear term of drying temperature was the most predominant factor influencing vitamin C content (p = 0.0010) . According to the Figure 2, it can be seen that by increasing of drying temperature vitamin C content increases significantly. The vitamin C content also increases with the increasing of vacuum pressure.

Total solids

Total solids in fresh blueberries can range from 13.4 to 15.6 g/100 g [6] up to 16.8 [18]. In vacuum dried sour cherries [19], the total solids were in the range from 32.52 to 86.47% (*i.e.*, 67.48–13.53% of moisture), depending on the drying conditions used.

Figure 2 shows that temperature had significant influence on total solids of dried blueberries. With the increase of temperature, the total solids increase also. This can be also confirmed by p-value (Table 3), where linear term of drying temperature (p = 0.0009) significantly influenced total solids, while other variables didn't show significant influence on total solids.

Total colour change

According to Mascan [20], total colour difference, ΔE, "which is a combination of parameters L-, a- and b--values, is a colorimetric parameter extensively used to characterise the variation of colors in foods during processing". If the difference between the samples is less than 1.0, it is assumed that difference would not be sensitively perceptible [21].

Total colour change (ΔE) of the vacuum dried blueberries varied from 88.33 to 91.88 which showed small difference in total colour change of dried samples. Liner terms of temperature (p = 0.0096) significantly affect the colour of the samples, while linear term of pressure, quadratic terms of investigated parameters and interaction between temperature and pressure didn't have a significant effect on total colour change (Table 3). Figure 2 shows surface plot for total colour change value as a function of temperature and pressure for the vacuum drying of blueberries. The plots indicate that ΔE values increased with increasing of drying temperature. It can be seen also that ΔE increased with increasing pressure up to about 300 mbar, while further increase show decrease of ΔE the dried blueberries.

Optimization of frozen blueberry vacuum drying process

The first goal for Response Surface Methodology is to find the optimum response. When there is more than one response then it is important to find the compromise optimum that does not optimize only one response [22]. The second goal is to understand how the response changes in a given direction by adjusting the design variables. The investigated response surface variables determine the quality of the product. The main goal of this research was to find the best settings for drying parameters, temperature and vacuum pressure. Desirability function was developed for the following criteria: maximum content of total phenols, vitamin C and antocyanin in dried blueberries and minimum total color change. By applying desirability function method, the optimum conditions of 60 °C and 100

mbar were obtained for vacuum drying of blueberries. At this optimum point, the calculated investigated responses were as follows: total phenolics 3.70 g CAE/ /100 g_{dw}, vitamin C 59.79 mg/100 g_{dw}, anthocyanin content 2746.33 mg/100 g_{dw}, total solids 89.50% and total colour change 88.83.

CONCLUSIONS

Response surface methodology was used for determining optimal drying temperature and vacuum pressure for drying process of frozen blueberries in terms of getting better final product quality. The total solids, total phenolic, vitamin C, anthocyanin content and total colour change were used as quality indicators of dried blueberries. The analysis of variance (ANOVA) showed that the regression models were statistically good with a significance level of $p < 0.05$ for all investigated responses. Considering the maximum amount of total phenolic content, vitamin C, antocyanin in dried blueberries as well as the minimum total colour change of the samples, the following optimum drying conditions were obtained: temperature of 60 °C and vacuum pressure of 100 mbar. The calculated responses were as follows: total phenols 3.70 g CAE/100 g_{dw}, vitamin C 59.79 mg/100 g_{dw}, anthocyanin content 2746.33 mg/ /100 g_{dw}, total solids 89.50%, and total colour change 88.83. Separate validation experiments were conducted at optimum conditions to verify predictions and adequacy of the second-order polynomial models. The experimental values agreed with those predicted, thus indicating the success of response surface methodology in optimizing the investigated drying conditions.

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IZVOD

OPTIMIZACIJA VAKUUM SUŠENJA ZAMRZNUTE DIVLJE BOROVNICE

Zdravko M. Šumić[1], Aleksandra N. Tepić[1], Stela D. Jokić[2], Radomir V. Malbaša[1]

[1]*Univerzitet u Novom Sadu, Tehnološki fakultet, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia*
[2]*Faculty of Food Technology, Osijek, Croatia*

(Naučni rad)

Cilj ovog istraživanja bila je optimizacija vakuum sušenja zamrznute borovnice primenom metode odzivne površine, u cilju očuvanja fitohemikalija. Sušenje je izvedeno u vakuum sušnici nove konstrukcije. Ispitani opseg temperatura i pritiska bio je 46–74 °C i 38–464 mbar. Kao pokazatelji kvaliteta sušene borovnice korišćeni su suva materija, ukupni fenoli, vitamin C, antocijani i ukupna promena boje. U okviru ispitanih parametara u eksperimentalnom opsegu, utvrđeni su optimalni uslovi vakuum sušenja borovnice na 60 °C i 100 mbar. Sprovedeni su odvojeni eksperimenti validacije u optimalnim uslovima u cilju verifikacije predviđanja i pogodnosti polinomskih modela drugog reda. U ovim optimalnim uslovima predviđena količina ukupnih fenola bila je 3,70 g CAE/100 g_{SM}, vitamina C 59,79 mg/100 g_{SM}, antocijana 2746,33 mg/100 g_{SM}, ukupna suva materija 89,50% i ukupna promena boje 88,83.

Ključne reči: Borovnica • Vakuum sušenje • Metoda odzivne površine • Kvalitet proizvoda