Technical paper

Optimization of Accelerated Solvent Extraction of Capsanthin from Red Paprika (*Capsicum annuum* L.) Using Response Surface Methodology

Ji-Sun KIM^{1,2}, Jong-Tae PARK³, Jiyun AHN², Tae-Youl HA² and Suna KIM^{4*}

¹Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, 145 Anam-ro, Seongbuk-gu, Seoul 02841, Republic of Korea

²Research group of Natural Materials and Metabolism, Korea Food Research Institute, 245 Nongsaengmyeong-ro, Iseo-myeon, Wanju-gun, Jeollabuk-do 55365, Republic of Korea

³Department of Food Science and Technology, Chungnam National University, 99 Daehak-ro, Yuseong-gu, Daejeon 34134, Republic of Korea

⁴Department of Food and Nutrition in Human Ecology, College of Natural Science, Korea National Open University, 86 Daehak-ro, Jongro-gu, Seoul 03078, Republic of Korea

Received July 9, 2018 ; Accepted January 14, 2019

In this study, accelerated solvent extraction (ASE) of capsanthin from red paprika was optimized by response surface methodology using a Box–Behnken design. Three independent variables (temperature, static time, and extraction solvent) were studied at 1 500 psi. As the response variable, capsanthin was quantitatively determined by ultra-performance liquid chromatography (UPLC) using a BEH C18 column after validating the capsanthin analysis method. The optimal extraction conditions were temperature of 100°C, static time of 5 min, and extraction solvent of 50 % acetone/ethanol (v/v). Under the optimized condition, the yield of capsanthin extraction was 26.86 ± 3.70 mg/100 g dry weight (dw), in good agreement with the predicted value (26.12 mg/100 g dw). Compared to conventional solvent extraction, the amount of organic solvent and required extraction time was reduced. This study provides technical guidance for the industrial extraction of capsanthin and improves the utilization of red paprika.

Keywords: red paprika, capsanthin, accelerated solvent extraction, response surface methodology, ultra-performance liquid chromatography

Introduction

Paprika (*Capsicum annuum* L.) is a widely used vegetable and food additive, as its fruit is considered a good source of carotenoid pigments (Kim *et al.*, 2011a; Kim *et al.*, 2009). Consumers consider paprika to be a very attractive vegetable because of its various colors, such as red, yellow, green, and orange, originating from carotenoids. Moreover, paprika contains various phytochemicals, including ascorbic acid, tocopherol, and flavonoids (Kim *et al.*, 2011b). In the *Capsicum* carotenoid biosynthesis pathway, capsanthin-capsorubin synthase synthesizes two red pigments, capsanthin and capsorubin (Deli *et al.*, 2001; Ha *et al.*, 2007; Guzman *et*

al., 2010), and capsanthin is the main carotenoid in red paprika. According to global market reports for carotenoids, capsanthin is one of five main products, along with β -carotene, lutein, astaxanthin, and annatto, whose production is expected to increase in food, feed, and cosmetic industries (März, 2015). Capsanthin acts by quenching $^1\text{O}_2$ and inhibiting lipid peroxidation caused by free radicals (Choe and Min, 2009; Maoka *et al.*, 2012). Capsanthin has 11 conjugated double bonds, including one conjugated carbonyl group (Fig. 1). The presence of a 3-hydroxy-*k*-end group with a conjugated carbonyl group, which is a characteristic structural moiety of paprika carotenoids, may enhance the activity of capsanthin for

*To whom correspondence should be addressed.

E-mail: ksuna7@knou.ac.kr

Fig. 1. Structure of capsanthin.

quenching $\cdot\text{OH}$ (Nishino *et al.*, 2016). In previous studies, we revealed that red paprika, with capsanthin as its main carotenoid, has protective effects against hydrogen peroxide-induced gap junction intercellular communication via suppression of reactive oxygen species formation (Kim *et al.*, 2016) and against diet-induced lipid accumulation (Kim *et al.*, 2017). However, although the importance of capsanthin as a functional, nutritional, and industrial material is well known, extraction of capsanthin is a time- and labor-consuming process that uses excessive amounts of organic solvent, such as chloroform, hexane, acetone, and petroleum ether (Arimboor *et al.*, 2015; Butnariu, 2016).

Carotenoids including capsanthin are unstable when exposed to light, oxygen, or high temperatures. So, careful handling in the laboratory is required to prevent the degradation and oxidation of the carotenoid pigment. All procedures should be conducted in subdued light and at low temperatures (Deli *et al.*, 2002). Conventional solvent extraction for carotenoids is soaked sample in organic solvent until colorless, and this procedure is required sufficient time and solvent. Because this extraction method takes a long time, the carotenoid pigments can be degraded or oxidized during the soaking time. Accelerated solvent extraction (ASE) is increasing mass transfer of extraction solvents by using elevated temperature and pressure. ASE can be applied to various extraction solvents to effectively save time and labor compared with other conventional solvent extraction techniques, such as Soxhlet extraction, ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction. Recently, various bioactive compounds, such as steviol glycosides from *Stevia rebaudiana* Bertoni leaves (Jentzer *et al.*, 2015), lignin from candlenut shells (Klein *et al.*, 2010), and anthocyanins from purple-fleshed sweet potato (Truong *et al.*, 2012), have been recovered using the ASE technique. ASE has also been applied for the extraction of contaminants, including phthalates, from environmental matrices (Khosravi and Price, 2015). Zaghdoudi *et al.* (2015) extracted lutein, zeaxanthin, β -cryptoxanthin, and β -carotene from kaki, peach, and apricot cultivated in Tunisia using ASE. Kang *et al.* (2016) extracted lutein and γ -tocopherol from paprika leaves and found a correlation between their

contents and ABTS activity. Saha *et al.* (2015) applied ASE for extraction of lutein and β -carotene from orange carrot. These results indicate the potential of ASE for fast and effective production of carotenoid concentrates for nutraceuticals or food colorants.

Response surface methodology (RSM) is an effective statistical and mathematical technique for experimental parameter optimization using experimental designs like Box–Behnken design (BBD), central composite design (CCD), and Doehlert's design (Zolgharnein *et al.*, 2013). Using an RSM model including these designs, the levels of the experimental parameters are identified and optimized to achieve an optimal response while performing a minimal number of runs (Jentzer *et al.*, 2015). Tsiaka *et al.* (2015) optimized extraction of carotenoids canthaxanthin, zeaxanthin, and lutein from *Aristeus antennatus* shrimp with UAE and MAE by employing a 16-run, 3-level BBD. Turan *et al.* (2015) investigated the effect of roasting on the functional properties of defatted hazelnut flour by utilizing a 10-run CCD in RSM. Jentzer *et al.* (2015) recovered steviol glycosides by ASE with an 18-run Doehlert's design in RSM. This study used the BBD, because it requires fewer runs in a 3-factor experimental design and it never includes extreme treatment combinations compared to all other RSM designs.

In the present study, we optimized ASE of capsanthin from red paprika using RSM by employing a BBD. Further, qualitative and quantitative analyses of capsanthin were performed after validating an ultra-performance liquid chromatography (UPLC) method for capsanthin analysis.

Materials and Methods

Materials Red paprika fruits (*Capsicum annuum* L., Var. Veyron) were grown in Kimje, South Korea, and harvested on 12 October 2013. The sample was kindly provided by Nongsan Trading Co. (Kimje, Jolla province, Korea). The sample was washed, drained, freeze-dried, ground, and then stored at -70°C for subsequent analysis. Capsanthin was purchased from ChromaDex (Irvine, USA) and β -apo-8'-carotenal (internal standard, IS) was purchased from Sigma-Aldrich Co. (St. Louis, USA). UPLC-grade methanol and acetone were purchased from Burdick & Jackson (Muskegon, USA). UPLC-

grade water was obtained by refining distilled water using an AquaMAX™-Ultra water purification system (YoungLin Instrument Co. Ltd., Seoul, Korea). All other chemicals were purchased from Junsei Chemical Co. Ltd. (Tokyo, Japan).

Capsanthin extraction For accelerated solvent extraction (ASE), sample was extracted with an ASE 150 system (Dionex, Sunnyvale, USA) equipped with 22-mL stainless steel cells and 60-mL collection vials. The powdered sample (1 g) was mixed with ASE Prep diatomaceous earth (Dionex) and loaded into an extraction cell containing a cellulose filter (ASE-NON-Stick Thimbles for extraction, Whatman, Schleicher & Schuell Bioscience, Dassel, Germany). The ASE conditions were as follows: static cycles, 3; solvent flush%, 60 volumes; nitrogen purge, 60 s; and pressure, 1 500 psi. Static time, temperature, and extraction solvent were used as extraction variables for ASE of capsanthin.

For comparison, conventional solvent extraction of capsanthin was performed according to a published method (Kim *et al.*, 2011a). In brief, exhaustive extraction with acetone was carried out using 1 g of powdered sample, which was placed in a bottle and impregnated with acetone at 4 °C in the dark. Extraction was performed for about 48 h with 160 mL acetone. All extracts were combined and evaporated to dryness using a TurboVap LV evaporator (Biotage, Uppsala, Sweden), and then redissolved in 3 mL of acetone. The acetone extract (3 mL) was incubated with 3 mL of methanol and 1 mL of 30 % potassium hydroxide in methanol at room temperature for 2 h 30 min in the dark, and then extracted three times with diethyl ether. The combined extract was then washed several times with distilled water until the pH was neutral. Subsequently, 5 mL of 10 % sodium chloride and 5 mL of 2 % sodium sulfate were added, and the separated hydrophilic phase was discarded. After evaporating the collected extracts, the residue was dissolved in acetone (1 mL) and stored at –20 °C under nitrogen until further use.

Capsanthin analysis by UPLC Capsanthin was analyzed using an Acquity UPLC H-Class system (Waters, Milford, USA) equipped with a UV detector (Tunable UV, TUV), column oven, sample manager (flow through needle injector, FTN), quaternary solvent manager (QSM), and Empower 3 chromatography software. Separation was performed using an Acquity UPLC BEH C18 column (2.1 × 50 mm, 1.8 µm) (Waters, Ireland) at 35 °C, and the mobile phase was a binary solvent system consisting of phase A (methanol) and phase B (water) with an isocratic program of 85 % A and 15 % B. The detection wavelength was set at 470 nm. The injection volume was 1.0 µL, and the flow rate was 0.5 mL/min.

Validation For method validation, stock solutions of capsanthin and β-apo-8'-carotenal (internal standard, IS) in dimethyl sulfoxide were prepared. The linearity of calibration, recovery ratio, precision, and accuracy were calculated to validate the UPLC method. To evaluate the linearity of sample detection, calibration curves were constructed with three

Table 1. Experimental range and values of the independent variables in the BBD for optimization of conditions for extraction of capsanthin from red paprika fruits using ASE.

X_i	Extraction condition	Level		
		–1	0	1
X_1	Temperature (°C)	60	80	100
X_2	Static time (min)	3	4	5
X_3	Acetone:Ethanol (v/v)	50:50	75:25	100:00

independent measurements of 12 capsanthin concentrations (range: 0.5–200 µg/mL) and the IS (50 µg/mL). The standard curve was constructed using linear regression of the peak area of capsanthin. The standard deviation and slope of the capsanthin calibration curve were used to calculate the limit of detection (LOD) and the limit of quantification (LOQ). To validate the recovery ratio, precision, and accuracy of the analytical method, within-run ($n = 5$) and between-run (5 d) analyses were performed using six different concentrations (10, 12.5, 25, 50, 75, and 100 µg/mL) of capsanthin within the linear range. The results were assessed by calculating the relative standard deviations (RSD %).

$$\text{LOD} = 3.3 \times (\text{standard deviation/slope of calibration curve})$$

$$\text{LOQ} = 10 \times (\text{standard deviation/slope of calibration curve})$$

$$\text{Recovery} = 100 \times (\text{observed content/original content})$$

Experimental design and statistical analysis Response surface methodology (RSM) was used to optimize capsanthin extraction from red paprika. ASE was considered to be influenced by three factors: temperature, static time, and extraction solvent (Table 1). A three-level, three-factor BBD (MINITAB Statistical Software, Release 14 for Windows, Minitab Inc., State College, USA) was applied to determine the best combination of extraction variables to maximize the yield of capsanthin extracted from red paprika fruits.

Experimental data were fitted to a quadratic polynomial model and regression coefficients were obtained. The nonlinear, computer-generated quadratic model used in the response surface was as follows:

$$Y = \beta_0 + \sum_{j=1}^k \beta_j X_j + \sum_{j=1}^k \beta_{jj} X_j^2 + \sum_{i < j} \beta_{ij} X_i X_j \quad \dots \text{Eq. 1}$$

where Y is the estimated response, β_0 , β_j , β_{jj} , and β_{ij} are the regression coefficients for intercept, linearity, square, and interaction, respectively, and X_i and X_j are independent coded variables. MINITAB software was used to estimate the response of each experimental design and set of optimized conditions. The fitness of the polynomial model equation was expressed by the coefficient R^2 . F- and p-values were used to check the significance of the regression coefficients. Data are expressed as the means of three replicate determinations.

Results and Discussion

Validation of UPLC method for capsanthin analysis As

Fig. 2. UPLC chromatogram of standard (A) and extract of red paprika fruit (B). Peak identification: (1) capsanthin and (2) β -apo-8'-carotenal (internal standard).

shown in Fig. 2, using UPLC and eluting with 85 % methanol, the retention time of capsanthin was 2.25 min, which is much shorter than the analytical time for other methods. For example, in a previous study, high-performance liquid chromatography (HPLC) analysis of capsanthin took about 12 min (Kim *et al.*, 2011a). To validate the UPLC method for capsanthin analysis, the LOD, LOQ, and linearity were determined, as shown in Table 2. The calibration curves were linear in the range of 0.5–200 $\mu\text{g/mL}$, and the mean regression equation of the calibration curves was $y = 11718x - 11417$, which showed good linearity ($R^2 = 0.9991$). The LOD and LOQ values were 1.2 and 3.7 $\mu\text{g/mL}$, respectively. In previous study, we conducted to 12 kinds of carotenoid analysis method using UPLC with an HSS-T3 column and gradient eluents of dimethylchloride/methanol/

acetonitrile and water. For validation of this method, we used only capsanthin as abundant compounds in red paprika. In 12 kinds carotenoid analysis method, LOD and LOQ values of capsanthin were 2.4 and 7.2 $\mu\text{g/mL}$, respectively (Hwang *et al.*, 2015). Even if validation for capsanthin has been performed, capsanthin analysis method performed in this study could be more appropriate for capsanthin analysis because short analysis time and simple isocratic eluent consisting methanol and water. The accuracy and precision of the UPLC analytical method for capsanthin were evaluated using a recovery study carried out at five concentration levels (Table 3). The recovery rates for this method were 91.88–99.23 % (within-run) and 97.32–104.23 % (between-run) with RSD values of 9.01 % or less. These results indicated that the analytical method shows good accuracy and

Table 2. LOD, LOQ, and linearity for capsanthin quantification by UPLC.

	Capsanthin
LOD ($\mu\text{g/mL}$) ^a	1.2
LOQ ($\mu\text{g/mL}$) ^b	3.7
Calibration equation ($y = Ax + B$)	
Slope (A)	11718
Intercept (B)	-11417
Correlation coefficient (R^2)	0.9991

^aLOD, limit of detection^bLOQ, limit of quantification

reproducibility for the measurement of capsanthin in red paprika.

Liu *et al.* (2011) compared the performance of UPLC and HPLC systems for lutein analysis and found that UPLC systems gave better results in terms of separation and analytical time. Previously, most chromatographic separations of capsanthin have been based on HPLC analysis using a C18 or C30 column, which is more time-consuming and uses a larger volume of solvent than UPLC analysis (Kim *et al.*, 2017; Murillo *et al.*, 2016).

Predicted model and statistical analysis Optimization of capsanthin extraction was performed using a BBD to examine ASE variables that could affect the extraction efficiency. BBD is a class of rotatable or nearly rotatable second-order designs based on three-level incomplete factorial designs. In addition, BBD does not contain combinations for which all factors are simultaneously at their highest or lowest levels. Ferreira *et al.* (2007) presented that BBD is a suitable design for RSM in terms of permitting such things as estimation of the quadratic model parameters, sequential design building, detection of lack-of-fit of the model, and using blocks. In this study, the extraction temperature (X_1 , 60–100 °C), static time (X_2 , 3–5 min), and acetone/ethanol ratio (X_3 , 50–100 % acetone) were examined. The whole design consisted of 15 experimental points, as listed in Table 4, and three replicates (run 15) at the center of the design were used to estimate the sum of squares

for pure error. The experiments were performed in triplicate at all design points in a randomized order. A 3D response surface plot generated by the model graphically represented the relationship between the independent and dependent variables (Fig. 3). Contour graph is the projection of 3D response surface plot on a 2D plot; it shows the maximum, minimum points as well as the effect of two parameters. Carotenoids are unstable at high temperature (Deli *et al.*, 2002). Shin *et al.* (1999) used Hunter α -values to estimate that the optimal condition for minimum degradation of red pigments in red paprika is a heating temperature 105 °C. The carotenoid structure, which consists of a long chain of conjugated carbon-carbon double bonds, is susceptible to light, oxygen, heat, and acid degradation. During heating, naturally occurring structures are disrupted and exposed to degradation, which leads to decreased color (Khachik *et al.*, 1986). For utilizing red paprika extraction or capsanthin, achieving the optimal conditions for minimizing the degradation of capsanthin as red pigment is very important. Therefore, we specified a range of extraction below 100 °C to avoid rigorous extraction.

The design matrix and the corresponding results of the RSM experiments for determining the effects of the three independent variables (acetone/ethanol ratio, extraction time, and extraction temperature) are shown in Tables 5 and 6. Through multiple regression analysis of the experimental data, the predicted response Y for the yield of capsanthin could be expressed by the following second-order polynomial equation in terms of the coded values:

$$Y = 18.09 + 2.48X_1 + 0.68X_2 + 0.85X_3 - 1.18X_1^2 + 2.05X_2^2 + 1.86X_3^2 - 1.39X_1X_2 - 1.93X_1X_3 - 2.45X_2X_3 \quad \dots \text{Eq. 2}$$

where Y is the yield of capsanthin (mg/100 g dry weight (dw)), and X_1 , X_2 , and X_3 are the coded variables for extraction temperature, static time, and acetone/ethanol ratio, respectively. Among the linear coefficients, only the static time was insignificant, and each of the quadratic coefficients was significant (Table 5). Statistical testing of the model was performed using analysis of variance (ANOVA), as shown in Table 6. The quadratic regression model showed a determination coefficient (R^2) of 0.8220 with no significant

Table 3. Within-run and between-run recoveries for capsanthin quantification by UPLC.

Analyte	Concentration ($\mu\text{g/mL}$)	Within-run ($n = 5$)		Between-run (5 d)	
		Recovery (%)	RSD (%) ^a	Recovery (%)	RSD (%)
Capsanthin	10	99.23	8.07	104.23	9.01
	12.5	94.45	6.46	102.81	5.57
	25	93.17	5.43	98.93	5.77
	50	91.88	5.15	97.32	2.86
	75	93.49	5.15	98.00	4.51
	100	94.72	5.87	97.68	3.16

^aRSD, relative standard deviation

Table 4. Factors and levels of the independent variables for RSM using BBD.

Order	Coded and uncoded variable levels			Yield of capsanthin (mg/100 g dw)	
	X_1 / temperature (°C)	X_2 / time (min)	X_3 / solvent (acetone %)	Experimental value	Predicted value
1	−1 (60)	−1 (3)	0 (75)	13.45 ± 0.99 ^a	14.41
2	−1 (60)	1 (5)	0 (75)	19.30 ± 1.94	18.55
3	1 (100)	−1 (3)	0 (75)	21.41 ± 0.04	22.15
4	1 (100)	1 (5)	0 (75)	21.69 ± 0.42	20.73
5	−1 (60)	0 (4)	−1 (50)	14.21 ± 0.78	13.51
6	−1 (60)	0 (4)	1 (100)	18.56 ± 2.19	19.07
7	1 (100)	0 (4)	−1 (50)	22.84 ± 2.73	22.33
8	1 (100)	0 (4)	1 (100)	19.45 ± 0.84	20.17
9	0 (80)	−1 (3)	−1 (50)	18.26 ± 0.87	18.02
10	0 (80)	−1 (3)	1 (100)	26.08 ± 0.24	24.62
11	0 (80)	1 (5)	−1 (50)	22.82 ± 0.11	24.28
12	0 (80)	1 (5)	1 (100)	20.85 ± 0.92	21.08
13	0 (80)	0 (4)	0 (75)	17.11 ± 0.65	18.09
14	0 (80)	0 (4)	0 (75)	19.05 ± 1.84	18.09
15	0 (80)	0 (4)	0 (75)	18.11 ± 2.82	18.09

^adata are mean ± standard deviation (SD), $n = 3$

lack-of-fit at 0.840 ($p > 0.05$), which means that the calculated model was able to explain 82.2 % of the results. Thus, the adequacy of the model was confirmed at the 0.000 % level of probability with R^2 and adjusted- R^2 values of > 80 %. No evidence for lack-of-fit of the model for any the response was indicated with a p -value > 0.05 .

Prediction and validation of optimal capsanthin extraction using the RSM model equation In this study, optimization was carried out to determine the conditions that maximized the extraction yield of capsanthin from red paprika. As shown Table 7, the optimal extraction conditions were an extraction temperature of 100 °C, a static time of 5 min, and an acetone/ethanol ratio of 50 % (v/v). To protect the carotenoids from high temperature mediated degradation, food samples are dehydrated using lyophilization such as freeze-drying. Therefore, carotenoid extraction is time consuming and using significant amount of solvent. The time lag between sample extraction and saponification are minimized to prevent enzymatic oxidation; also, a short extraction time with appropriate temperature is recommended for viable extraction. Saini and Keum (2018) suggest that ASE can replace the time-consuming Soxhlet extraction for industrial scale extraction. Under these optimal conditions, the predicted model value of 26.12 mg/100 g dw is in good agreement with the experimental value of 26.86 ± 3.70 mg/100 g dw. In previous studies, capsanthin samples have been obtained by repeating solvent extraction until the solvent is colorless as conventional solvent extraction (Kim *et al.*, 2011a). Hence, it is necessary to compare the yields of capsanthin obtained by ASE and

conventional solvent extraction. The capsanthin yields obtained using conventional solvent extraction (26.73 ± 0.48 mg/100 g dw) and ASE (26.86 ± 0.70 mg/100 g dw) were no statistically significant difference. However, the time and amount of solvent required for ASE (30 min and 50 mL, respectively) were only 1.042 % and 31.25 % of those required for conventional solvent extraction (48 h and 160 mL, respectively).

Traditional solvent extraction methods are often time-consuming and laborious; also, they have low selectivity or extraction yields, and require large amounts of organic solvents (Saha *et al.*, 2015). As carotenoids are sensitive to light, heat, oxygen, and acid (Butnariu 2016a), it is important to minimize the extraction time. The use of ASE can save considerable time and reduce the amount of solvent compared with conventional solvent extraction.

In the present study, we showed the efficiency of capsanthin recovery from red paprika using ASE designed by RSM comparing with conventional solvent extraction, which is time, labor, and excessive organic solvents consuming process. The results revealed that ASE is an effective technique for capsanthin extraction in red paprika. Considering that capsanthin has various functional values, mass production of capsanthin using ASE would promote the availability of capsanthin as health enhancing materials as well as food colorants. Furthermore, we reduced the usage of organic solvents to 33 % using ASE under high pressure and high temperature. It meant that ASE for recovering capsanthin could be an effective extraction technique for polluting less by organic solvents as well as for producing functional food

(A)

(B)

(C)

Fig. 3. Response surface and contour plots for the effects of extraction conditions on capsanthin content of red paprika fruits using ASE: Temperature and solvent (A); Time and solvent (B); Temperature and time (C).

Table 5. Regression coefficients of predicted quadratic polynomial model for different responses.

Term	Coefficient	T-value	p-value
Constant	18.09	32.579	0.000
Temperature	2.48	7.303	0.000
Static time	0.68	2.003	0.053
Extraction solvent ratio	0.85	2.508	0.017
Temperature × Temperature	-1.18	-2.360	0.024
Static time × Static time	2.05	4.104	0.000
Extraction solvent ratio × Extraction solvent ratio	1.86	3.708	0.001
Temperature × Static time	-1.39	-2.892	0.007
Temperature × Extraction solvent ratio	-1.93	-4.022	0.000
Static time × Extraction solvent ratio	-2.45	-5.091	0.000

Table 6. Variance analysis of the regression parameters in the predicted response surface quadratic model.

Source	DF ^a	SS ^b	MS ^c	F-value	p-value
Regression	9	419.422	46.602	16.79***	0.000
Linear	3	176.604	58.868	21.21***	0.000
Square	3	102.780	34.260	12.34***	0.000
Interaction	3	140.037	46.679	16.82***	0.000
Residual error	33	91.583	2.775	-	-
Lack of fit	27	66.482	2.462	0.59	0.840
Pure error	6	25.102	4.184	-	-
Total	44	515.303	-	-	-

^aDF, degree of freedom^bSS, sum of squares^cMS, mean square**Table 7.** Optimal conditions and predicted and experimental values under optimal conditions.

Response	Capsanthin
Optimal conditions	
Temperature (°C)	100
Static time (min)	5
Solvent (Acetone:Ethanol, v/v)	50:50
Predicted value (mg/100 g dw)	26.12
Experimental value for ASE (mg/100 g dw)	26.86 ± 3.70 ^a
Experimental value for conventional solvent extraction (mg/100 g dw)	26.73 ± 0.48

^aData are mean ± standard deviation (SD), *n* = 3

materials.

Acknowledgments This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Golden Seed Project, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) [grant number 213006-05-3-WTX11]; and the Korea Food Research Institute (KFRI).

References

- Arimboor, R., Natarajan, R. B., Menon, K. R., Chandrasekhar, L. P., and Moorkoth, V. (2015). Red pepper (*Capsicum annuum*) carotenoids as a source of natural food colors: analysis and stability - a review. *J. Food Sci. Tech.*, **52**, 1258–1271.
- Butnariu, M. (2016). Methods of analysis (extraction, separation, identification and quantification) of carotenoids from natural products. *J. Ecosyst. Ecogr.*, **6**, 1–19.

- Choe, E. and Min, D. B. (2009). Mechanisms of antioxidants in the oxidation of foods. *Compr. Rev. Food Sci. Food Saf.*, **8**, 345–358.
- Deli, J., Molnár, P., Matus, Z., and Tóth, G. (2001). Carotenoid composition in the fruits of red paprika (*Capsicum annuum* var. *lycopersiciforme rubrum*) during ripening; biosynthesis of carotenoids in red paprika. *J. Agric. Food Chem.*, **49**, 1517–1523.
- Deli, J. and Molnár, P. (2002). Paprika carotenoids: analysis, isolation, structure elucidation. *Curr. Org. Chem.*, **6**, 1197–1219.
- Ferreira, S. C., Bruns, R., Ferreira, H., Matos, G., David, J., David, G., Brandao, E. P., da Silva, L., Portugal, P., Reis, D., and Souza, A. (2007) Box-Behnken design: an alternative for the optimization of analytical methods. *Anal. Chim. Acta*, **597**, 179–186.
- Guzman, I., Hamby, S., Romero, J., Bosland, P. W., and O'Connell, M. A. (2010). Variability of carotenoid biosynthesis in orange colored *Capsicum* spp. *Plant Sci.*, **179**, 49–59.
- Ha, S. H., Kim, J. B., Park, J. S., Lee, S. W., and Cho, K. J. (2007). A comparison of the carotenoid accumulation in *Capsicum* varieties that show different ripening colours: deletion of the capsanthin-capsorubin synthase gene is not a prerequisite for the formation of a yellow pepper. *J. Exp. Bot.*, **58**, 3135–3144.
- Hwang, J. R., Hwang, I. K., and Kim, S. (2015). Quantitative analysis of various carotenoids from different colored paprika using UPLC. *Korean J. Food Sci. Technol.*, **47**, 1–5.
- Jentzer, J. B., Alignan, M., Vaca-Garcia, C., Rigal, L., and Vilarem, G. (2015). Response surface methodology to optimise accelerated solvent extraction of steviol glycosides from *Stevia rebaudiana* Bertoni leaves. *Food Chem.*, **166**, 561–567.
- Kang, J. H., Kim, S., and Moon, B. (2016). Optimization by response surface methodology of lutein recovery from paprika leaves using accelerated solvent extraction. *Food Chem.*, **205**, 140–145.
- Khachik, F., Beecher, G. R., and Whittaker, N. F. (1986). Separation, identification, and quantification of the major carotenoid and chlorophyll constituents in extracts of several green vegetables by liquid chromatography. *J. Agric. Food Chem.*, **34**, 603–616.
- Khosravi, K. and Price, G. W. (2015). Determination of phthalates in soils and biosolids using accelerated solvent extraction coupled with SPE cleanup and GC–MS quantification. *Microchem. J.*, **121**, 205–212.
- Kim, J. S., Ahn, J., Lee, S. J., Moon, B., Ha, T. Y., and Kim, S. (2011a). Phytochemicals and antioxidant activity of fruits and leaves of paprika (*Capsicum annuum* L., var. Special) cultivated in Korea. *J. Food Sci.*, **76**, C193–C198.
- Kim, J. S., Ahn, J. Y., Ha, T. Y., Rhee, H. C., and Kim, S. (2011b). Comparison of phytochemical and antioxidant activities in different color stages and varieties of paprika harvested in Korea. *Korean J. Food Sci. Technol.*, **43**, 564–569.
- Kim, J. S., Lee, W. M., Rhee, H. C., and Kim, S. (2016). Red paprika (*Capsicum annuum* L.) and its main carotenoids, capsanthin and β -carotene, prevent hydrogen peroxide-induced inhibition of gap-junction intercellular communication. *Chem-Biol. Interact.*, **25**, 146–155.
- Kim, J. S., Ha, T. Y., Kim, S., Lee, S. J., and Ahn, J. (2017). Red paprika (*Capsicum annuum* L.) and its main carotenoid capsanthin ameliorate impaired lipid metabolism in the liver and adipose tissue of high-fat diet-induced obese mice. *J. Funct. Food*, **31**, 131–140.
- Kim, S., Ha, T. Y., and Hwang, I. K. (2009). Analysis, bioavailability, and potential healthy effects of capsanthin, natural red pigment from *Capsicum* spp. *Food Rev. Int.*, **25**, 198–213.
- Klein, A. P., Beach, E. S., Emerson, J. W., and Zimmerman, J. B. (2010). Accelerated solvent extraction of lignin from *Aleurites moluccana* (candlenut) nutshells. *J. Agric. Food Chem.*, **58**, 10045–10048.
- Liu, H., Zhang, Y., Li, Q., Zou, Y., Shao, J., and Lan, S. (2011). Quantification of lutein and zeaxanthin in marigold (*Tagetes erecta* L.) and poultry feed by ultra-performance liquid chromatography and high performance liquid chromatography. *J. Liq. Chromatogr. Relat. Technol.*, **34**, 2653–2663.
- März, U. (2015). FOD025C–The global market for carotenoids. *BCC Research*.
- Maoka, T., Tokuda, H., Suzuki, N., Kato, H., and Etoh, H. (2012). Anti-oxidative, anti-tumor-promoting, and anti-carcinogenesis activities of nitroastaxanthin and nitrolutein, the reaction products of astaxanthin and lutein with peroxynitrite. *Mar. Drugs*, **10**, 1391–1399.
- Murillo, E., Turcsi, E., Szabo, I., Mosquera, Y., Agócs, A., Nagy, V., Gulyás-Fekete, G., and Deli, J. (2016). Carotenoid composition of the fruit of red mamey (*Pouteria sapota*). *J. Agric. Food Chem.*, **64**, 7148–7155.
- Nishino, A., Yasui, H., and Maoka, T. (2016). Reaction of paprika carotenoids capsanthin and capsorubin with reactive oxygen species. *J. Agric. Food Chem.*, **64**, 4786–4792.
- Saha, S., Walia, S., Kundu, A., Sharma, K., and Paul, R. K. (2015). Optimal extraction and fingerprinting of carotenoids by accelerated solvent extraction and liquid chromatography with tandem mass spectrometry. *Food Chem.*, **177**, 369–375.
- Saini, R. K. and Keum, Y. S. (2018) Carotenoid extraction methods: A review of recent developments. *Food Chem.*, **240**, 90–103.
- Shin, J. H., Rheem, S., Seo, J. K., Lee, S. K., Shim, J. H., Kim, S. K., and Baek, Y. J. (1999). Optimizing minimum degradation condition of red pigments in red bell pepper (*Capsicum annuum* L.) using response surface methodology. *Food Sci. Biotechnol.*, **8**, 351–356.
- Truong, V. D., Hu, Z., Thompson, R., Yencho, G. C., and Pecota, K. (2012). Pressurized liquid extraction and quantification of anthocyanins in purple-fleshed sweet potato genotypes. *J. Food Compos. Anal.*, **26**, 96–103.
- Tsiaka, T., Zoumpoulakis, P., Sinanoglou, V. J., Makris, C., Heropoulos, G. A., and Calokerinos, A. C. (2015). Response surface methodology toward the optimization of high-energy carotenoid extraction from *Aristeus antennatus* shrimp. *Anal. Chim. Acta*, **877**, 100–110.
- Turan, D., Capanoglu, E., and Altay, F. (2015). Investigating the effect of roasting on functional properties of defatted hazelnut flour by response surface methodology (RSM). *LWT-Food Sci. Technol.*, **63**, 758–765.
- Zaghdoudi, K., Pontvianne, S., Framboisier, X., Achard, M., Kudaibergenova, R., Ayadi-Trabelsi, M., Kalthoum-Cherif, J.,

-
- Vanderesse, R., Frochot, C., and Guiavarch, Y. 2015. Accelerated solvent extraction of carotenoids from: Tunisian Kaki (*Diospyros kaki* L.), peach (*Prunus persica* L.) and apricot (*Prunus armeniaca* L.). *Food Chem.*, **184**, 131–139.
- Zolgharnein, J., Shahmoradi, A., and Ghasemi, J. B. (2013). Comparative study of Box–Behnken, central composite, and Doehlert matrix for multivariate optimization of Pb (II) adsorption onto Robinia tree leaves. *J. Chemometr.*, **27**, 12–20.