Waste and Biomass Valorization
https://doi.org/10.1007/s12649-021-01431-z

ORIGINAL PAPER

Bioactive Compounds Extraction from the Black Carrot Pomace with Assistance of High Pressure Processing: An Optimization Study

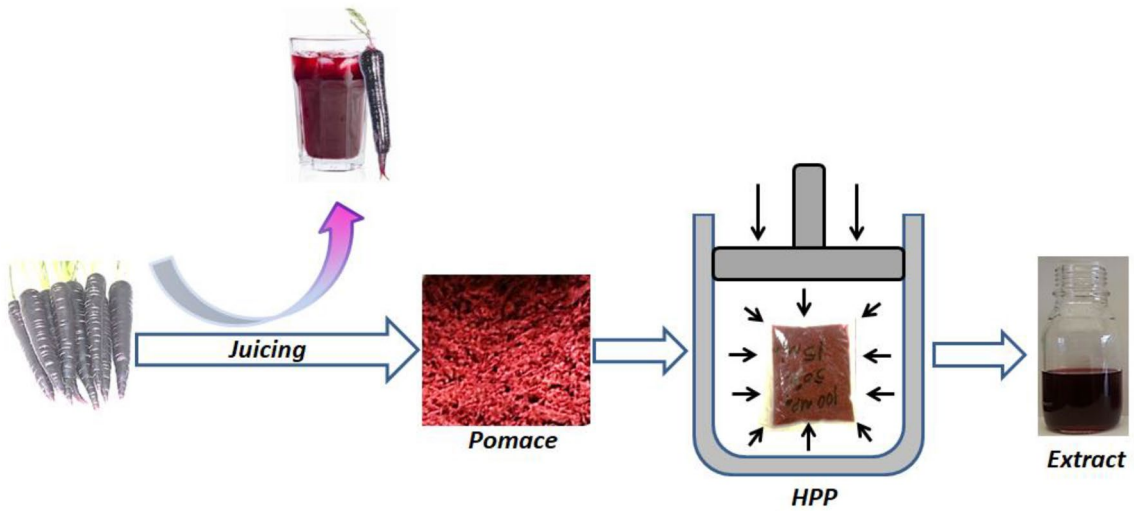
E. Agcam[1] · A. Akyıldız[1] · S. Kamat[2] · V. M. Balasubramaniam[2,3]

Received: 26 October 2020 / Accepted: 17 March 2021
© The Author(s), under exclusive licence to Springer Nature B.V. 2021

Abstract

The aim of the presented study was to determine the ability of the high pressure processing (HPP) for extraction of bioactive compounds from black carrot pomace and to optimize treatment conditions (pressure, temperature, processing time) for obtaining the maximum yield of bioactive compounds. Experiments were conducted using pilot scale high pressure food processors and water as the extraction solution. A set of response surface analysis mathematical models were developed and validated for the extraction of bioactive compounds of black carrot pomace, considering total monomeric anthocyanin contents (TMA), total phenolic compounds (TPC) and antioxidant activity (AA). Quality attributes such as total color difference (ΔE*) and polymeric color ratio (PCR) of the samples were also evaluated. The validation results showed that the error values of the obtained models were lower than 10%, which means acceptable error value in modelling study. Combined pressure–thermal treatment at 266.6 MPa, 78.3 °C for 13.6 min was identified as the optimal process condition for maximum extraction of bioactive compounds.

Graphic Abstract



Keywords Black carrot pomace · High pressure processing · Bioactive compounds extraction · Response surface methodology · Optimization

✉ E. Agcam
eagcam@cu.edu.tr

Extended author information available on the last page of the article

Published online: 26 March 2021

Statement of Novelty

An alternative extraction methodology was developed with assistance of high pressure processing to improve mass transfer of sensitive bioactive compounds from the black carrot pomace. Recovering of bioactive compounds of black carrot pomace was optimized for high pressure processing conditions by aid of response surface methodology. The results revealed that high pressure assistance extraction is a promising green technology to collect valuable bioactive compounds, especially from waste product of juice industry. This green technology, in addition to increase extraction yield of bioactive compounds, was able to reduce extraction time, solvent consumption, bioactive compounds degradation, and environmental pollution caused by toxic solvents. Therefore, in the near future, HPP technology has a high potential to be used as assistant in bioactive compounds extraction from plant/animal sources.

Introduction

Carrots are ancient crops that have been used as vegetable based food source since it was first cultivated at least 3000 years ago in Eastern Region. In today's world, there are two main types of cultivated carrots: the first one is called Eastern or anthocyanin carrots (*Daucus carota* L. ssp. *sativus* var. *atrorubens* Alef.) due to its purple/black color and second one is called Western or carotene carrots (*Daucus carota* L. ssp. *sativus* var. *sativus*) due to its orange color [1]. The black carrot is a cultivar traditionally grown in Turkey, Afghanistan, Egypt, Pakistan and India, so those countries are the most important producers of it. In 2017, it was produced higher than 120 kilo tons and generally processed to the juice, juice concentrate and fermented beverage called shalgam in Turkey [2, 3].

The black carrot is a source of cyanidin-based anthocyanin compounds which are able to contribute consumers' health by reducing risks of heart diseases, stroke, and tumors as well as by improving anti-inflammatory effects and cognitive behavior. Moreover, the potential usage of anthocyanin compounds as natural food colorants is remarkably increasing because of consumer concerns of synthetic food colorants. The black carrot juice and concentrate were successfully used as both enrichment and color of foods in cake [4], fresh-cut apple [5], several fruit juices and nectars [6], strawberry jam [7], and candies [8].

Extraction is a sufficient method used for collecting pure component or components from a solid or liquid mixture. There are many extraction methods, such as heat reflux, soxhlet, boiling, agitating, soaking and distilling, which are traditional; and ultrasonic, microwave, pulsed electric fields, supercritical fluid and high pressure, which are emerging extraction assistance technologies [9–11]. Nowadays, in order to reduce costs and environmental pollution, the expectations from novel extraction assistance techniques are to shorten processing times, reduce solvent consumption, and increase extraction efficiency [11].

High pressure processing (HPP, 100–800 MPa) is defined as a food preservation method which involves subjecting food to intense pressure with or without assistance of heat, to achieve microbial inactivation while achieving consumer-desired qualities. HPP causes a little effect on covalent bonds and, thus, the processed food does not undergo significant chemical changes. Therefore, small molecules in food product such as flavor compounds, vitamins, and pigments are typically not affected by high pressure treatment [10, 12]. Recent studies have shown that high pressure processing can be also used to enhance mass transfer rates, which increases cell permeability as well as secondary metabolite diffusion [13–17]. The extraction with assistance of high pressure has been recently applied to extract bioactive compounds from plant materials, such as, anthocyanins from grape [18], corilagin and phenolic compounds from longan fruit pericarp [13, 19], phenolic compounds from litchi fruit pericarp [14], ginsenosides from ginseng [16], antioxidant compounds from papaya [17], and finally phenolic compounds from pansies [20]. Prasad et al. [21] reported that high pressure extraction technique performed a higher efficiency than ultrasonic and conventional extraction techniques in extracting phenolic compounds from litchi fruit pericarp, and they concluded that it is able to reduce time of the extraction and increase the extraction yield of phenolic compounds. Zhang et al. [22] compared different extraction techniques in terms the yield of ginsenosides and extraction time. The study revealed highest yield through high pressure extraction at 500 MPa-2 min, followed by ultrasonic assisted extraction for 30 min, refluxed for 4 h and supercritical CO_2 for 4 h. It was reported that the high pressure extraction is coming up with the following advantages: time saving, higher extraction yields, cleaner extracts (especially lower or without toxic solvents), minimal heat based bioactive compounds degradation in comparison with classical extraction technologies [20].

The earlier studies showed that the pomace obtained after juicing of black carrot retains considerable level of bioactive compounds (especially anthocyanin compounds) [3, 23]. Therefore, the main objectives of presented study are to put forth the ability of the high pressure processing for extraction of bioactive compounds from black carrot pomace, to obtain mathematical models related with those compounds and high pressure processing conditions, and finally to optimize the process conditions for obtaining the black carrot

pomace extract with the highest yield of bioactive compounds as well as meeting with adequate quality attributes.

Material and Methods

Black Carrot Pomace

The black carrot pomace was supplied by local juicing company (TARGID, Mersin, Turkey) as waste product of production of black carrot juice concentrate. The black carrot pomace packaged with polyethylene bags under vacuum was stored at − 65 °C until the study conducted, in order to eliminate undesirable reactions.

The Handling of Black Carrot Pomace

The black carrot pomace was shipped from Turkey to USA under frozen (− 30 °C) conditions and stored frozen until used. The pomace was firstly diluted with pure water at ratio of 1:3 (w/w). Pure water served as a non-toxic, low cost and environmentally safe extraction solvent to recover the bioactive compounds from the black carrot pomace. After that section, the black carrot pomace mix was obtained. The black carrot mix (150 g) was filled to small pressure resistance polypropylene bags and vacuum sealed to obtain sample pouches. The experiments are carried out at The Ohio State University, Emerging Food Technology Pilot plant, Columbus, OH.

High Pressure Treatment

The obtained pouches were loaded into a cylindrical stainless steel canister (11.0 cm × 65.5 cm) insulated with 0.5 cm polytetrafluoroethylene (PTFE) and filled with propylene glycol (Brenntag Mid-South, Inc., St. Louis, MO). The canister was then loaded into the pilot plant pressure chamber of a Stansted Iso-Lab FPG11500 (Stansted Fluid Power LTD, Stansted, Essex, UK) included temperature controlling and maintenance system. The produced hydrostatic pressure was transmitted by a food-grade polypropylene glycol (Brenntag Mid-South, Inc., St. Louis, MO). The samples were preconditioned at the desired initial temperature taking into account the final process conditions as well as the heat of compression [24]. Temperature of the samples was monitored inside the sample holder using a brass stuffing box (Ecklund-Harrison, FortMyers, FL) and a *T*-type thermocouple (Omega Engineering, Inc., Stamford, CT). Representative graph related to pressure and temperature of the sample during processing time was shown in Fig. 1.

Following to HPP treatment for each runs, the obtained sample pouches were quickly immersed into the ice bath to cool down them. After that the processed samples were loaded into the 50 mL falcon tubes and then centrifuged at 4000 rpm, 4 °C for 20 min (Heraeus Bofuge Primo R, Germany) to collect supernatants used in the analyses. Finally, the collected supernatants were stored at − 24 °C until analyses. All the analyses were performed in 2 days in order to eliminate storage effect.

Experimental Design of High Pressure Treatments

Response surface methodology (RSM) was used to design experimental conditions and to optimize the extraction parameters. A central composite design (CCD) was employed in this regard. Pressure (*P*), temperature (*T*) and time (*t*) were chosen for independent variables. The experimental design consists of 20 experiments including 8 factorial and 6 axial points, and 6 replicates of the central point. The coded and actual levels of three independent variables

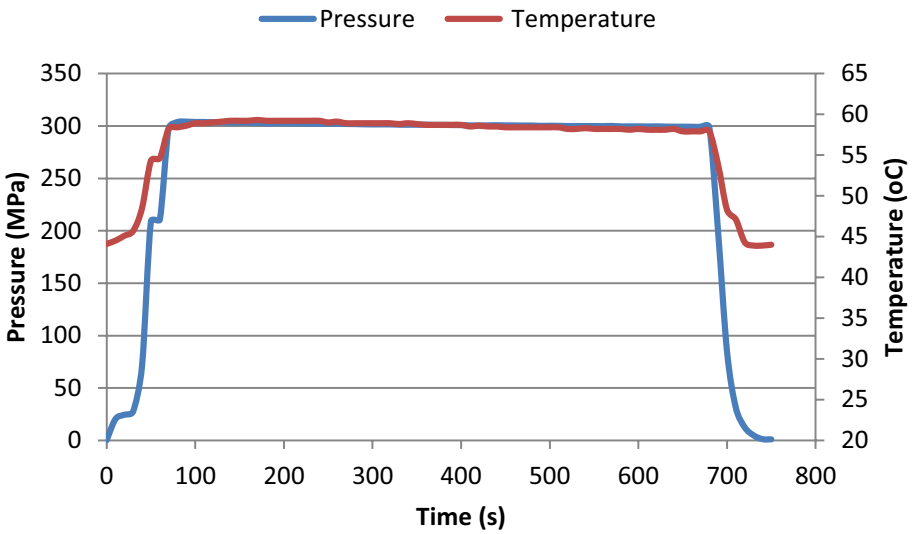


Fig. 1 The representative figure of pressure–temperature history

Table 1 Coded and actual levels of three variables

Independent variables	Coded levels				
	− 1.68	− 1	0	+ 1	+ 1.68
Pressure (*P*, MPa)	0.1	121.7	300.1	478.4	600
Temperature (*T*, °C)	25	38.2	57.5	76.8	90
Time (*t*, min)	0	4.1	10	15.9	20

were given in Table 1. Total monomeric anthocyanin content (TMA), anthocyanin compounds, total phenolic content (TPC), antioxidant activity (AA), polymeric color ratio (PCR) and total color difference (ΔE*) were selected as the responses (or dependent variables) for the combination of the independent variables. To eliminate unexpected variability in the observed responses, the runs related with experimental conditions were randomized. Design Expert software (Version 10, Stat-Ease, Inc., Minneapolis, MN, USA) was used for analysis of the experimental design data, optimization of high pressure assisted extraction conditions, and calculation of predicted responses.

Statistical Evaluation of the Models

The independent variables of high pressure treatments were coded with α-values and the statistical analysis of the models were carried out according to those coded values. The significance level of the mathematical models and terms of them were determined by variance analysis (ANOVA). The model adequacies were checked in terms of the values of R^2, *adjusted*-R^2 and *predicted*-R^2. In addition, the model that has insignificant *p*-value ($p > 0.05$) for *lack-of-fit* test was selected for prediction study. Terms of models which have higher *p*-value than 0.1 ($p > 0.1$) were removed and then the statistical analysis was repeated. Term reduction study was started from the model terms which have the highest *p*-value. After term reduction, when the model performance outputs (especially, *adjusted*-R^2 and *predicted*-R^2) improved, the related term was removed from the mathematical model.

The optimization study was conducted according to the obtained models with certain limitations. While desired quality parameters were aimed to maximize, undesired ones were aimed to minimize. Then, numerical solution was carried out in order to calculate optimum extraction conditions with assistance of high pressure processing. The desirability function (*D*), a simple mathematical method, was used to determine the optimum process conditions.

$$D = (d_1 \times d_2 \times d_3 \times \ldots \times d_m)^{\frac{1}{m}} = \left(\prod_{i=1}^{m} d_i \right)^{\frac{1}{m}} \quad (1)$$

where d_i is desirability value for any response and m is response number. The solution that has the highest desirability value was chosen as optimum process conditions for independent variables.

RSM Model Validation

After developing mathematical models, the models were validated by conducting additional independent experiments at different process conditions (100 MPa–50 °C–15 min, 300 MPa–70 °C–5 min and 500 MPa–90 °C–1 min) and samples were analyzed as outlined in earlier sections. The prediction accuracy of the models was then determined in terms of relative error value related with actual and predicted outputs for the same process conditions.

$$Error\,(\%) = \left| \frac{X_{predcted} - X_{actual}}{X_{actual}} \right| \times 100 \quad (2)$$

where $X_{predicted}$ is dependent value obtained from model equation and X_{actual} is dependent value obtained from experimental condition.

Determination of Total Phenolic Content (TPC)

The TPC of the samples were measured with some modification of the Folin-Ciocalteu method previously described by Abdullakasim et al. [25]. For the analysis, 100 µL supernatant collected from treated pomace transferred into the glass test tubes and then mixed thoroughly with 100 µL Folin-Ciocalteu reagent and 3000 µL deionized water. Following incubation of the tubes for 10 min at room temperature, 100 µL of 20% Na_2CO_3 solution was added with immediate mixing and incubated further to equilibrate the reactions at room temperature for 2 h in the darkness. The absorbance measurement was performed at 765 nm with using of a spectrophotometer (Perkin Elmer Lambda 25-UV/VIS, USA). The calibration curve was prepared by making of several gallic acid concentrations and then those concentrations were analyzed accordingly with protocols mentioned above. The total phenolic contents of the extracts were expressed in milligram gallic acid equivalents per L (mgGAE/L). All total phenolic content measurements were carried out as five replicates.

Determination of total monomeric anthocyanin content (TMA)

Total monomeric anthocyanin content was determined using with the pH differential method described by Giusti, Wrolstad [26]. Monomeric anthocyanin pigments undergo reversible structural transformations with the function of pH. While the colored oxonium form predominates at pH

1.0, the colorless hemiketal form predominates at pH 4.5. This basic reaction triggered by medium pH is the basis of the pH-differential method and, permits accurate and rapid determination of the total monomeric anthocyanins [26]. For measurement of the TMA, 0.5 mL supernatant was added to two glass tubes and then to one of them 9.5 mL pH = 1.0 potassium chloride solution (0.025 M) to another one 9.5 mL pH = 4.5 sodium acetate solution (0.4 M) were added and shacked. The dilutions were then allowed to equilibrate for 15 min at room temperature (~ 22 °C). The absorbance of the equilibrated samples was measured against a blank cell containing distilled water with 1 cm path length disposable cuvettes at 520 ($\lambda_{\text{vis-max}}$) for anthocyanin content and at 700 nm for haze correction. The absorbance (A) of the diluted sample was then calculated as follows:

$$A = \left(A_{\lambda_{\text{vis - max}}} - A_{700\text{ nm}}\right)_{\text{pH=1}} - \left(A_{\lambda_{\text{vis - max}}} - A_{700\text{ nm}}\right)_{\text{pH=4.5}} \tag{3}$$

The total monomeric anthocyanin content in the sample was calculated according to the following formula:

$$\text{TMA (mg/L)} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times l} \tag{4}$$

in which cyanidin-3-glucoside molecular weight (MW = 449.2 gmol^{-1}), the dilution factor (DF),the molar absorptivity constant (ε = 29,600 L cm^{-1} mol^{-1}) and l = path length (1 cm) were used. Results were expressed as mg of cyanidin-3-glucoside equivalents per L of extract. All spectrophotometric analyses were performed using a UV–visible spectrophotometer (Perkin Elmer Lambda 25 UV/VIS, MA, USA). All total monomeric anthocyanin measurements were replicated five times.

Antioxidant Activity (AA)

The antioxidant activity of the samples was evaluated by DPPH* (1,1-diphenyl-2-picrylhydrazyl) free radical-scavenging method. The DPPH* free radical-scavenging activity measurements were carried out according to the previously followed procedure of Klimczak et al. [27] with some modifications. Briefly, 0.1 mL of black carrot pomace extract was added to 3 mL of DPPH* radical (0.025 gL^{-1} in 80% methyl alcohol) and mixed by vortex. The absorbance of the samples was measured at 515 nm using the spectrophotometer after waiting 60 min in the dark for equilibrium the reaction. The antioxidant activity was expressed as the percentage of decline of the absorbance.

$$AA\,(\%) = \left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \times 100 \tag{5}$$

where $A_{control}$ is the absorbance of the control, and A_{sample} is the absorbance of the sample. All antioxidant activity measurements were replicated three times.

Color

The color analysis was performed with HunterLab equipment (Hunter Associates Laboratory, Inc., Reston, VA, USA). The color parameters were collected as L*, a* and b* parameters. L*, a* and b* values represent brightness, red (+ a*) to green (− a*) color and yellow (+ b*) to blue (− b*) color, respectively [28]. Total color difference (ΔE*) value was calculated by following formula wit using of L*, a* and b* parameters for treated and untreated samples.

$$\Delta E* = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \tag{6}$$

Pigment Degradation

In order to determine a value related with anthocyanin degradation, the polymeric color ratio (PCR) was calculated for the black carrot pomace extracts. The pigment degradation of extracts was determined by previous method presented by Giusti, Wrolstad [26]. In that analysis, the dilution factor previously applied in the monomeric anthocyanin analysis was used. Shortly, 0.5 mL pomace extract and 9.5 mL pure water were added to the glass tube and then vortexed to obtain stock solution of the extracts. 2.8 mL of the stock solution was added to two different glass tubes. 0.2 mL pure water was added to one of the tubes and 0.2 mL potassium metabisulfite solution ($K_2S_2O_5$, w/v, 20%) to the other. Both tubes were stored in the dark for 15 min to equilibrate. The absorbance of equilibrated samples was collected at 420, 520 and 700 nm with UV–visible spectrophotometer (Perkin Elmer Lambda 25 UV/VIS, MA, USA). All analyses were replicated three times.

While anthocyanin compounds react with bisulfite to form colorless sulfonic acid complexes, anthocyanin-tannin complexes and melanoidins are resistant to bleaching by bisulfite. Thus, by treating bisulfite to the sample, a representative value for brown pigments can be obtained with the absorbance collected at 420 nm. The color density value is defined as the sum of absorbances at the $\lambda_{\text{vis-max}}$ (520 nm) and 420 nm for the samples untreated with bisulfite (Eq. 8), whereas the polymeric color value is defined as sum of the absorbances for the samples treated with it (Eq. 9). Moreover, the ratio of the color density and polymeric color values can be used to determine the PCR parameter that is useful for understanding the contribution of the polymerized compounds on the sample color (Eq. 10). The rising in the PCR value means high degradation in the monomeric anthocyanin and formation of brown pigments [26].

The following equations were used for calculation of PCR values:

$$\text{Color density} = \left[\left(A_{\lambda_{\text{vis - max}}} - A_{700\text{ nm}}\right) + \left(A_{420\text{ nm}} - A_{700\text{ nm}}\right)\right]_{water\ aded} \times DF \tag{8}$$

$$\text{Polimeric color} = \left[\left(A_{\lambda_{\text{vis - max}}} - A_{700\text{ nm}}\right) + \left(A_{420\text{ nm}} - A_{700\text{ nm}}\right)\right]_{bisulfite\ added} \times DF \tag{9}$$

$$PCR = \frac{PC}{CD} \times 100 \tag{10}$$

where *PCR* is polymeric color ratio value, *PC* is polymeric color value and *CD* is color density value.

Results and Discussion

Mathematical Models

The experimental study results given in Table 2 are used to determine the best fitting mathematical models for TMA, TPC, AA and ΔE*, PCR of black carrot pomace extracts. The ANOVA outputs for the responses and the estimated term coefficients of the mathematical models were presented in Supplementary Tables (Table S1 and S2).

The best model performances are obtained as 2FI for TMA content of black carrot pomace extracts ($p < 0.0001$). All the terms for 2FI-TMA model were determined significant with exception of *Pt* term. Because of *Pt* interaction term reduction, the model performance outputs decreased. Therefore, it is decided that *Pt* term must be in the 2FI equation, although it has insignificant *p*-value (Eq. 11). As a result, 2FI-TMA model can explain relationship between independent variables [pressure (*P*), temperature (*T*) and processing time (*t*)] and dependent parameter (TMA) with insignificant *lack-of-fit* value ($p = 0.7702$) as well as 0.9664, 0.9510 and 0.9031 values of R^2, *adjusted*-R^2 and *predicted*-R^2, respectively.

$$TMA(\text{mg/L}) = 79.817 + 0.00986068P + 0.43807T + 0.69493t - 0.000268299PT - 0.000468462Pt - 0.012388Tt \tag{11}$$

TPC values of black carrot pomace extracts were analyzed in order to determine suitability of different mathematical models. According to the results, the highest performance outputs was obtained for 2FI model ($p < 0.0001$). In this situation, it is decided to use the model of 2FI for definition of relationship between different *P*–*T*–*t* and TPC values (Eq. 12). Then, elaborated statistical analysis was conducted on 2FI-TPC model and the results showed that while *Tt* interaction term was insignificant ($p > 0.1$), the remaining terms were identified as significant ($p < 0.1$). After elimination of *Tt* term, R^2, *adjusted*-R^2 and *predicted*-R^2 values of the model improved remarkably, and the values were calculated as 0.9782, 0.9685 and 0.9452; respectively. And also, *lack-of-fit* of the 2FI-TPC model was determined insignificant with the $p = 0.3463$.

$$\begin{aligned} TPC\ (\text{mg/L}) = {} & 309.924 - 0.150P + 0.089828T \\ & - 0.053321t + 0.00220418PT \\ & + 0.00320844Pt \end{aligned} \tag{12}$$

The AA results were tested for different models and according to the essential model performance markers, the best model was quadratic model for prediction of AA capacity of the extracts obtained from the black carrot pomace ($p < 0.0001$). Thus, the quadratic model was chosen to determine effectiveness of *P*, *T* and *t* on AA results of the obtained extracts (Eq. 13). In addition, all quadratic terms were reached significant values except quadratic term of the pressure (P^2). The term was removed and the reduced model terms were analyzed again to obtain clearer predictor equation. After term reduction, the model improved and R^2, *adjusted*-R^2, and *predicted*-R^2 values of the model were calculated as 0.9477, 0.9096 and 0.7255, respectively. Further, *lack-of-fit* value was reached up to 0.3000 for reduced quadratic model of the AA yield. Therefore, all remaining terms of the reduced model were significant after elimination of P^2 term ($p < 0.1$).

$$\begin{aligned} AA\ (\%) = {} & 88.73711 + 0.000328659P + 0.13814T \\ & - 0.56981t - 0.0000700278PT \\ & + 0.000503933Pt + 0.00588093Tt \\ & - 0.00169704T^2 + 0.00636524t^2 \end{aligned} \tag{13}$$

The color quality is important for final product and one of the best representative color value is total color change (ΔE*) after processing. Different mathematical models were tested for ΔE* values of the collected extracts and the results showed that quadratic model had the best performance ($p < 0.0001$). While all of the liner, quadratic and *PT* from interaction terms were determined significant in ΔE* quadratic model ($p < 0.1$), *Pt* and *Tt* interaction terms were determined insignificant with *p*-values of 0.7446 and 0.8023, respectively. The term reduction process was applied to the *Pt* and *Tt* terms of the ΔE* quadratic model,

Table 2 Experimental conditions and response values for different process combinations

Run	Independent variables			Responses									
				TMA (mg/L)		TPC (mg/L)		AA (%)		ΔE*		PCR (%)	
	Pressure (*P*, MPa)	Temperature (*T*, °C)	Time (*t*, min)	Actual	Predicted	Actual	Predicted	Actual	Predicted	Actual	Predicted	Actual	Predicted
U	–	–	–	67.97 ± 1.27		220.58 ± 6.13		75.09 ± 2.85		–		9.54 ± 0.07	
1	121.7 (− 1)	76.8 (+ 1)	4.1 (− 1)	111.95 ± 2.48	110.89	324.11 ± 4.42	320.54	88.95 ± 0.86	88.59	4.27 ± 0.03	4.21	10.97 ± 0.61	10.90
2	121.7 (− 1)	38.2 (− 1)	15.9 (+ 1)	99.81 ± 3.55	99.13	312.44 ± 9.24	310.71	88.35 ± 0.17	88.34	4.48 ± 0.04	5.56	12.66 ± 1.02	12.24
3	600.0 (+ 1.68)	57.5 (0)	10.0 (0)	99.00 ± 3.12	98.68	321.20 ± 10.10	319.85	90.21 ± 0.52	90.19	12.94 ± 0.02	12.88	29.79 ± 1.84	27.64
4	300.1 (0)	57.5 (0)	10.0 (0)	102.95 ± 3.14	101.76	317.07 ± 7.23	317.20	90.02 ± 0.91	89.79	5.50 ± 0.07	5.40	17.88 ± 1.24	18.74
5	300.1 (0)	57.5 (0)	10.0 (0)	100.27 ± 2.34	101.76	321.59 ± 11.72	317.20	90.01 ± 0.53	89.79	5.96 ± 0.09	5.40	17.75 ± 1.10	18.74
6	478.4 (+ 1)	76.8 (+ 1)	15.9 (+ 1)	101.19 ± 3.35	100.66	350.93 ± 9.36	349.70	90.79 ± 0.70	90.50	13.86 ± 0.13	13.22	35.88 ± 1.46	35.86
7	300.1 (0)	57.5 (0)	10.0 (0)	101.40 ± 1.47	101.76	315.52 ± 12.67	317.20	89.50 ± 0.28	89.79	5.45 ± 0.05	5.40	19.83 ± 0.63	18.74
8	121.7 (− 1)	38.2 (− 1)	4.1 (− 1)	97.72 ± 2.24	97.16	305.62 ± 7.62	306.71	90.05 ± 0.39	90.21	3.73 ± 0.11	4.28	17.41 ± 0.63	17.35
9	300.1 (0)	57.5 (0)	0.0 (− 1.68)	102.86 ± 4.24	103.34	307.50 ± 9.79	308.11	89.81 ± 0.55	89.96	2.21 ± 0.36	1.69	16.43 ± 0.51	16.62
10	478.4 (+ 1)	38.2 (− 1)	15.9 (+ 1)	96.36 ± 3.06	96.32	304.36 ± 11.08	305.47	90.15 ± 0.71	90.37	4.96 ± 0.10	5.31	18.60 ± 0.68	18.60
11	300.1 (0)	25.0 (− 1.68)	10.0 (0)	93.63 ± 3.70	94.16	295.38 ± 8.03	292.79	88.89 ± 0.63	88.62	5.83 ± 0.13	4.23	15.52 ± 0.55	14.20
12	300.1 (0)	57.5 (0)	10.0 (0)	100.14 ± 2.49	101.76	316.88 ± 5.73	317.20	89.94 ± 0.33	89.79	4.24 ± 0.10	5.40	18.94 ± 0.66	18.74
13	478.4 (+ 1)	76.8 (+ 1)	4.1 (− 1)	106.78 ± 1.04	106.38	332.81 ± 3.98	332.08	87.65 ± 0.81	87.52	12.26 ± 0.03	11.94	25.36 ± 1.50	25.70
14	121.7 (− 1)	76.8 (+ 1)	15.9 (+ 1)	107.90 ± 2.55	107.16	326.71 ± 10.85	324.55	89.53 ± 0.91	89.43	6.09 ± 0.09	5.49	14.38 ± 0.62	13.34
15	300.1 (0)	57.5 (0)	10.0 (0)	102.50 ± 2.08	101.76	316.61 ± 8.60	317.20	89.49 ± 1.19	89.79	6.32 ± 0.05	5.40	17.80 ± 1.27	18.74
16	0.1 (− 1.68)	57.5 (0)	10.0 (0)	104.12 ± 1.92	104.83	311.43 ± 10.14	314.56	89.37 ± 0.58	89.39	7.01 ± 0.21	6.59	10.29 ± 0.98	9.85
17	300.1 (0)	90.0 (+ 1.68)	10.0 (0)	108.16 ± 1.70	109.35	338.16 ± 6.42	341.62	86.92 ± 1.32	87.37	9.69 ± 0.06	10.82	23.85 ± 0.70	23.29
18	300.1 (0)	57.5 (0)	10.0 (0)	101.76 ± 1.85	101.76	315.92 ± 7.27	317.20	89.63 ± 0.73	89.79	4.84 ± 0.02	5.40	17.42 ± 0.72	18.74
19	478.4 (+ 1)	38.2 (− 1)	4.1 (− 1)	96.69 ± 2.61	96.35	285.84 ± 5.74	287.85	90.14 ± 0.42	90.10	3.04 ± 0.20	4.02	15.02 ± 0.71	15.98
20	300.1 (0)	57.5 (0)	20.0 (+ 1.68)	99.93 ± 2.08	100.18	324.02 ± 3.41	326.30	90.86 ± 0.13	90.90	3.80 ± 0.05	3.84	19.11 ± 1.44	20.87

U is untreated sample extracted at room temperature (23.5 ± 1.5 °C). The extracts for U were collected to eliminate time effect which was independent parameter during extraction, as soon as black carrot mix had been obtained. Predicted values were calculated according to mathematical model equations. n = 5 for TMA and TPC, n = 3 for AA, ΔE and PCR

and then the statistical analyses were repeated. The model performance outputs were improved after insignificant terms reduction and R^2, *adjusted*-R^2, *predicted*-R^2 and *lack-of-fit* values of the model were calculated as 0.9487, 0.9187, 0.7954 and 0.2407, respectively (Eq. 14).

$$\Delta E * = 13.69926 - 0.051746P - 0.30389T + 0.63451t + 0.000579341PT + 0.0000481807P^2 + 0.00201313T^2 - 0.026335t^2 \quad (14)$$

The PCR results obtained for experimental design fitted with the highest performance for 2FI model ($p < 0.0001$). All the *p*-values of the model terms were remarkably calculated lower than 0.1 ($p << 0.1$). Therefore, effect of high pressure processing conditions on PCR of black carrot pomace extracts was mathematically represented with all 2FI model terms (Eq. 15). For the 2FI-PCR model; R^2, *adjusted*-R^2, *predicted*-R^2 and *lack-of-fit* values were respectively determined as 0.9748, 0.9631, 0.9400 and 0.2303 (Eq. 15).

$$CR\,(\%) = 34.81693 - 0.055974P - 0.37622T - 1.27837t + 0.00117255PT + 0.00182114Pt + 0.016426Tt \quad (15)$$

Effect of HPP Conditions on Phenolic Contents and Quality Attributes

The mathematical model equations formed by experimental design of HPP conditions, in order to explain relationships between responses and independent variables, were used to figure out effectiveness of HPP as extraction assistance technology. The results showed that TMA contents of high pressure processed extracts ranged between 93.63 and 111.95 mg/L. The best model for TMA results was already determined 2FI model used to obtain 2D and 3D graphs relating TMA and HPP conditions. The relationship between HPP conditions and TMA was shown in Fig. 2. While TMA yield did not change significantly with increasing pressure, increasing sharply with higher processing temperature at the constant processing time. However, at the situation of both higher processing pressure and time, the TMA yield would decrease linearly at the constant processing temperature. Finally, if processing pressure was constant, the TMA yield would increase slightly linear for higher processing time and a sharp linear for higher processing temperature. As a result, it can be said that in order to obtain the highest black carrot anthocyanins, HPP conditions must be chosen as high temperature and low processing pressure and time.

The TPC values of the black carrot pomace extracts varied in the range between 284.84 and 350.93 mg/L. Effects of processing conditions on TPC yield can be represented graphically with using of designed TPC-2FI mathematical model. Model graphs showed that when temperature is low and pressure is increasing at certain processing time, TPC value is decreasing linearly up to the highest pressure point. However, TPC model expected that if temperature and pressure were high together, the TPC yield would be higher due to synergistic effect (Fig. 3A). Similar synergic effect was obtained for pressure and time changing at constant processing temperature (Fig. 3B). At the constant processing pressure, the TPC yield is expected to increase linearly by the model for both time and temperature increasing. The maximum TPC yield would be obtained for the range of 20–14 min processing time and 80–90 °C processing temperature at the constant pressure (Fig. 3C).

The AA values of the HPP treated extracts were changed between 87.65 and 90.86%. The graphs obtained by reduced quadratic model for experimental results of AA were shown in Fig. 4. The graphs showed that linear change was expected by the model and higher AA value would be obtained for the pomace extracts processed at higher pressure and constant processing temperature and time (Fig. 4A). However, at constant pressure and time, AA would increase gradually up to ~ 55 °C saturation point and then start to decrease with similar tendency. The AA yield model at constant processing temperature is expected that while the pomace extract with the highest AA values are collected at ranges of 300–600 MPa and 15–20 min, the lowest extracts are collected at ranges of 500–600 MPa and 0.1–3 min (Fig. 4B). As can be seen in Fig. 4C, the process conditions ranged between 25 and 50 °C and 0.1–3 min as well as 30–90 °C and 10–20 min could be defined the zone of the highest and moderate AA values.

The ΔE* graph outputs of the reduced quadratic model were shown in Fig. 5. For rising of ΔE* in the extracts, the synergic effect was observed between processing pressure and temperature at the constant treatment time. Thus, the highest ΔE* values are expected for pressure and temperature conditions, in ranges of 400–600 MPa and 60–90 °C, respectively (Fig. 5A). The data suggested that, while the ΔE* value could increase up to ~ 10 min and after that point could gradually decrease at the constant pressure and temperature, the ΔE* value could gradually increase without saturation point for increasing of processing pressure at the constant time and temperature (Fig. 5B). The resemble ΔE* changing tendencies were predicted in response to processing time and temperature changings at constant pressure (Fig. 5C).

The PCR model showed that PCR value of the extracts would remarkably increase for extreme processing pressure, temperature and time. The PCR value would response with linear increasing the process conditions for increasing

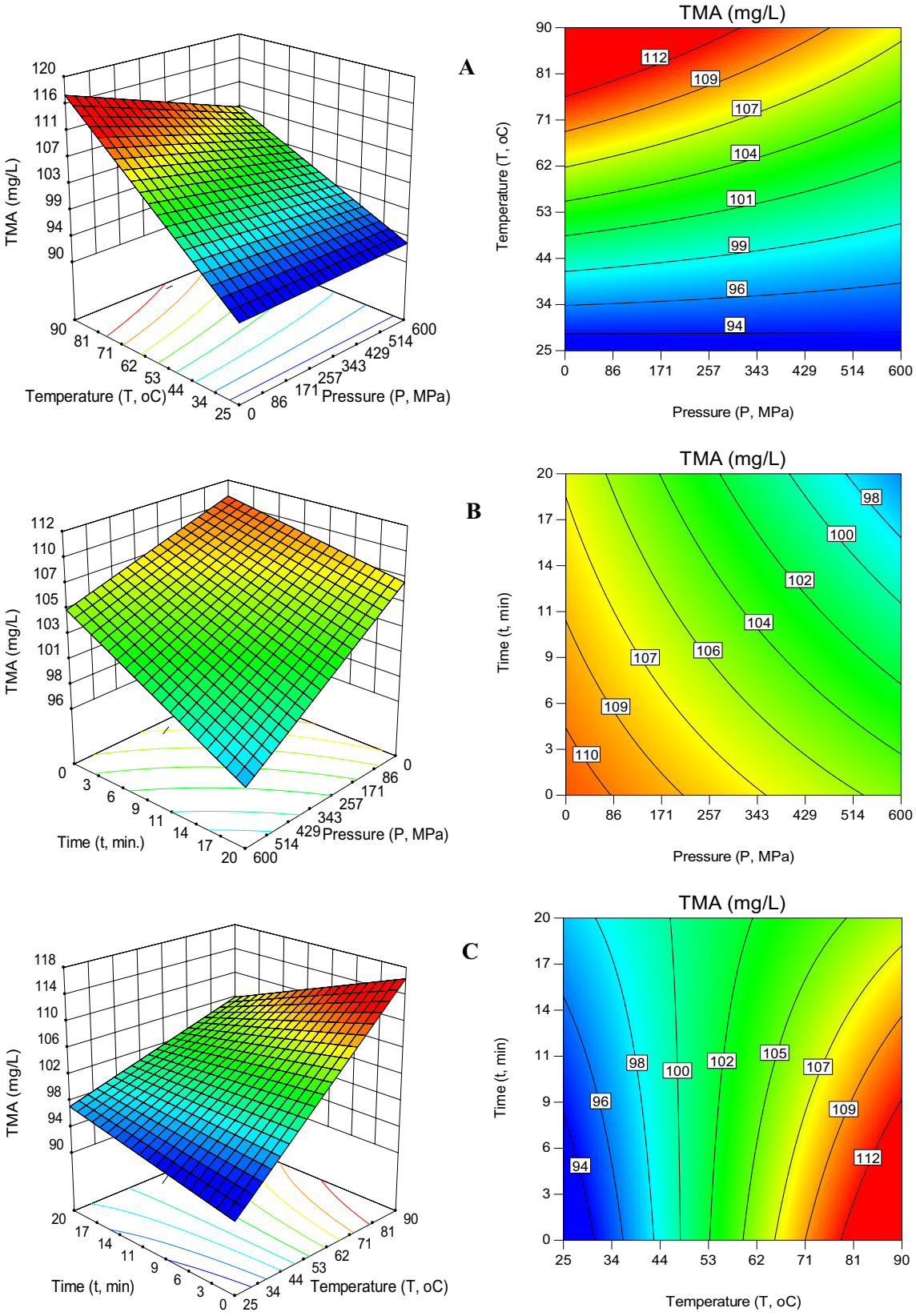


Fig. 2 Effect of pressure, temperature and processing time on total monomeric anthocyanin contents (TMA) yield of black carrot pomace extract-3D graphs and counter plots (**A** $t = 5$ min, **B** $T = 70$ °C and **C** $P = 200$ MPa)

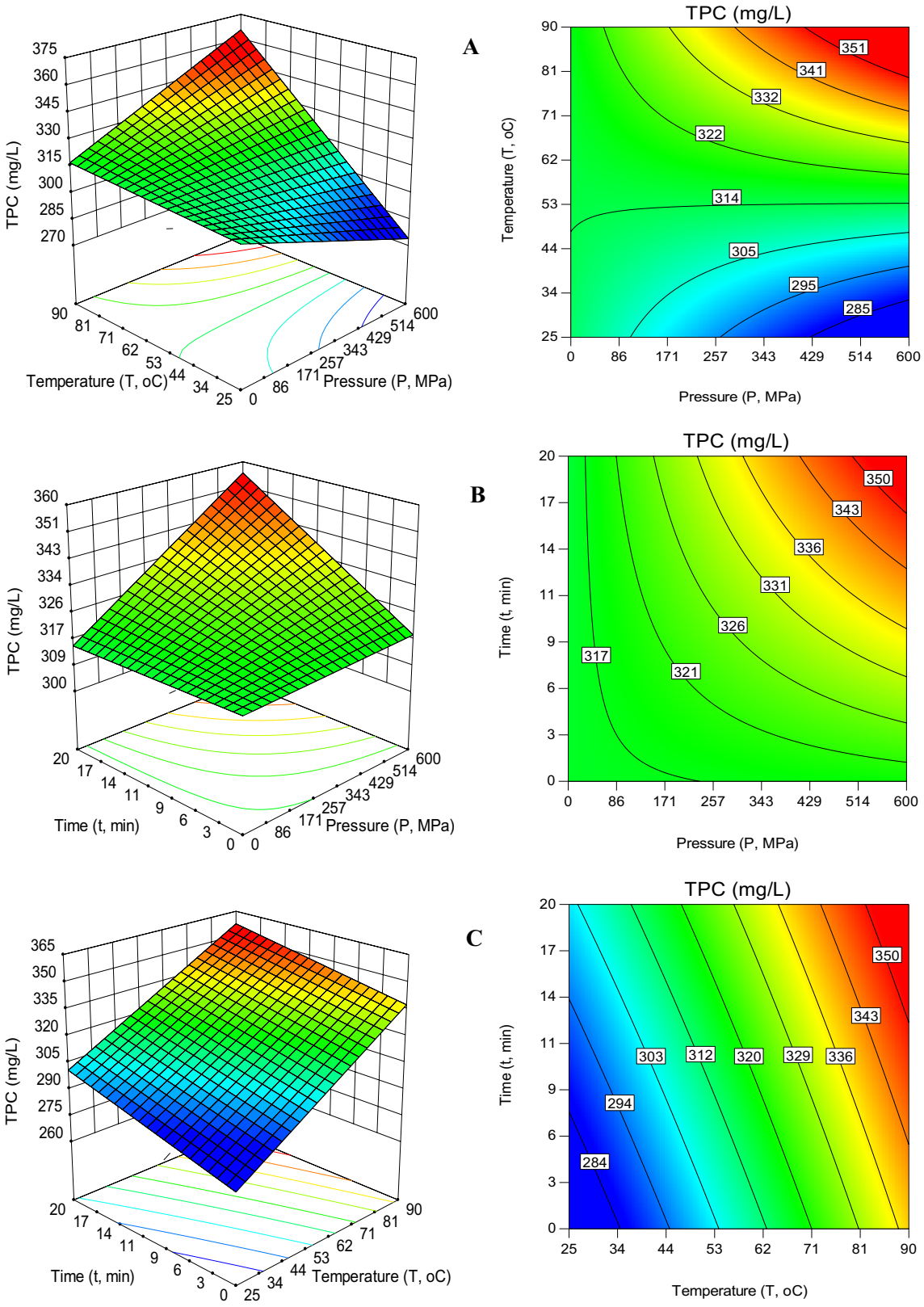


Fig. 3 Effect of pressure, temperature and processing time on total phenolic content (TPC) yield of black carrot pomace extract-3D graphs and counter plots (**A** $t = 10$ min, **B** $T = 70$ °C and **C** $P = 375$ MPa)

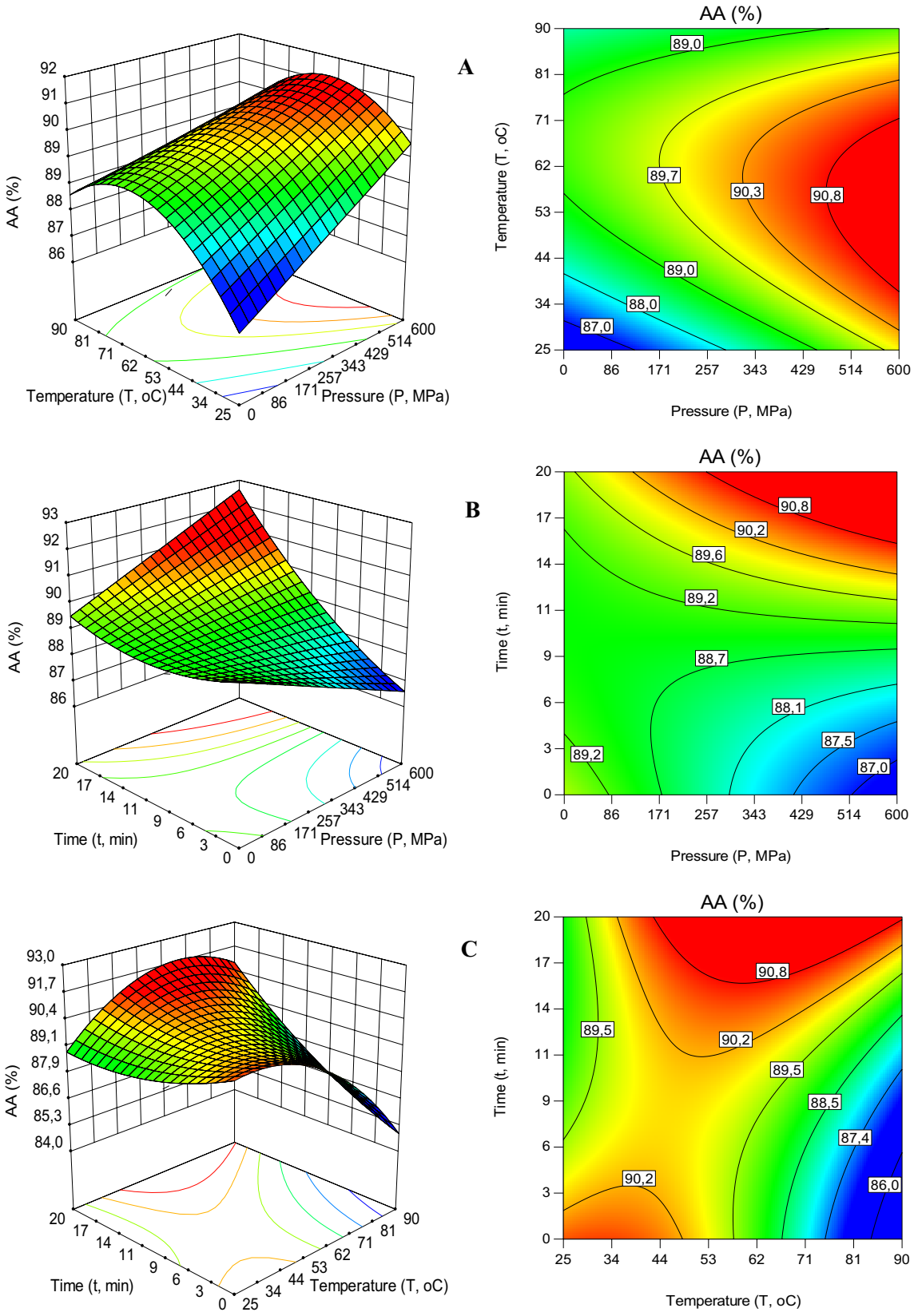


Fig. 4 Effect of pressure, temperature and processing time on antioxidant activity (AA) yield of black carrot pomace extract-3D graphs and counter plots (**A** $t = 15$ min, **B** $T = 75$ °C and **C** $P = 425$ MPa)

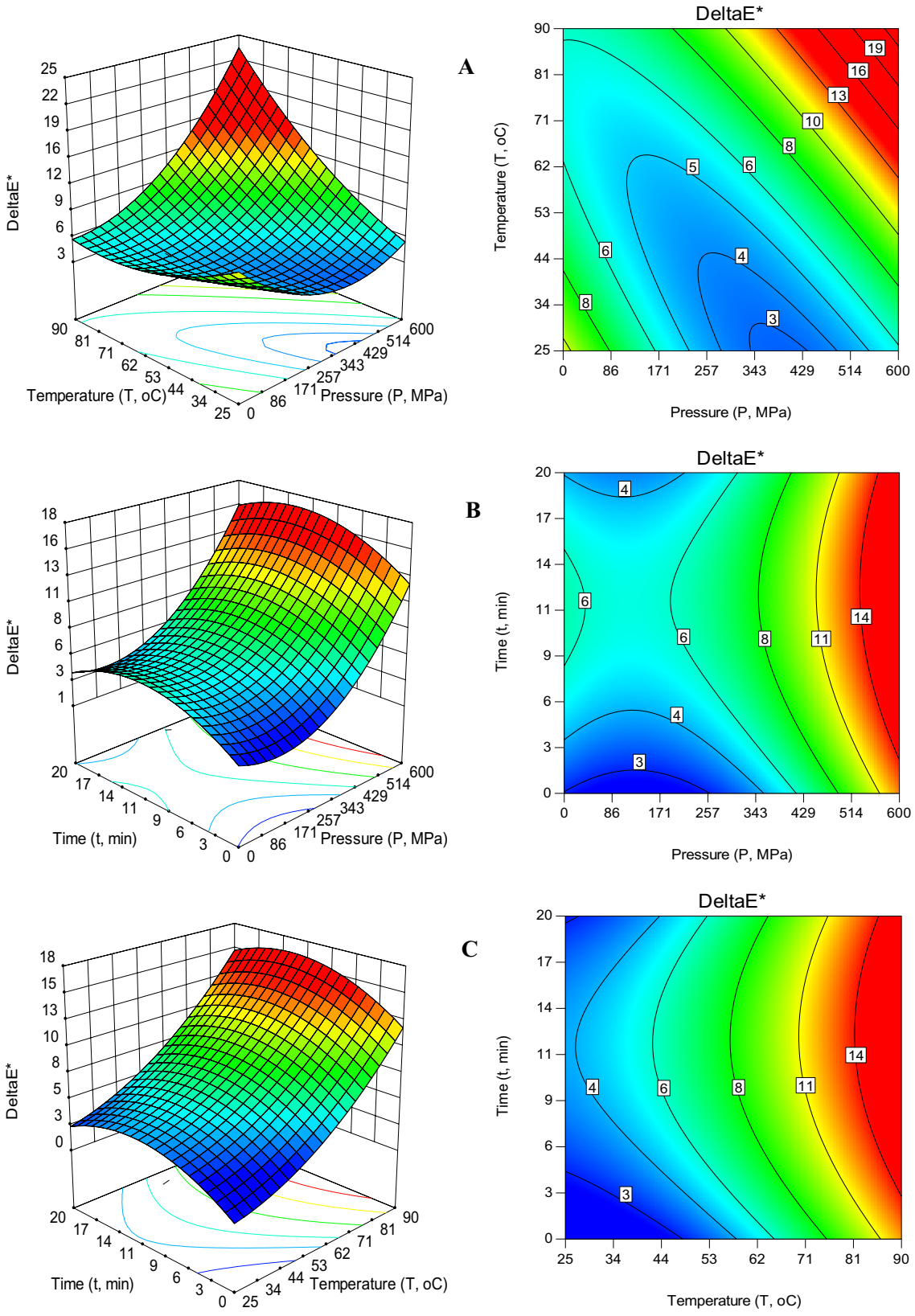


Fig. 5 Effect of pressure, temperature and processing time on total color difference (ΔE*) value of black carrot pomace extract-3D graphs and counter plots (**A** $t = 7$ min, **B** $T = 70$ °C and **C** $P = 450$ MPa)

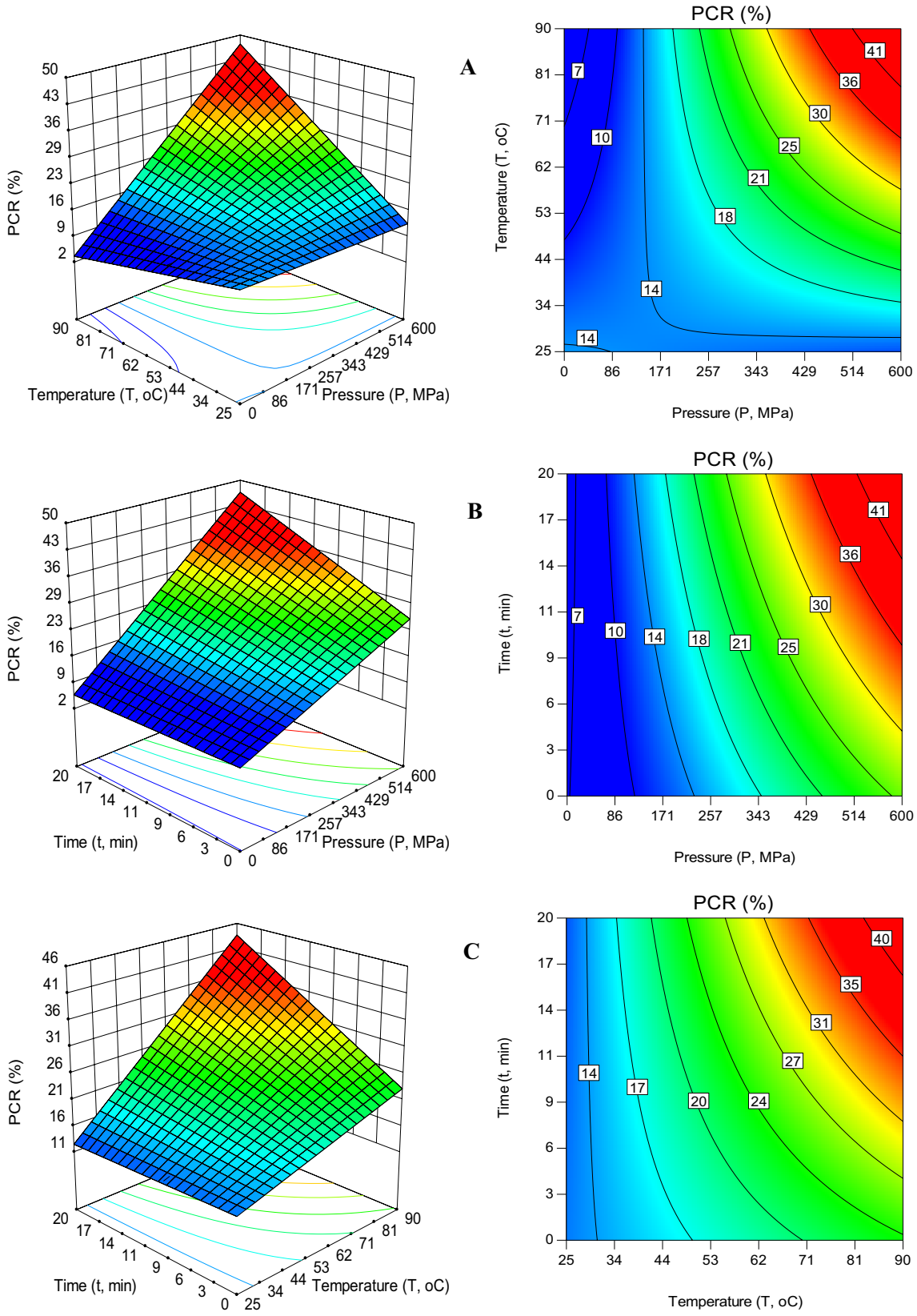


Fig. 6 Effect of pressure, temperature and processing time on polymeric color ratio (PCR) value of black carrot pomace extract-3D graphs and counter plots (**A** $t = 13$ min, **B** $T = 75$ °C and **C** $P = 450$ MPa)

pressure at constant processing time and temperature, while not changing meaningfully for higher time at constant processing pressure and temperature (Fig. 6). Moreover, the model predicted that undesirable PCR values could be obtained for extracts processed at 450–600 MPa pressure, 70–90 °C temperature and 5–20 min.

Validation

Initially, the process parameters used to calculate the models' performances must be chosen for the validation study. In presented study, for taking into consideration of all the model spaces defining the high and low levels of independent variables, three different pressures (100, 300 and 500 MPa), temperatures 50, 70 and 90 °C) and processing times (1, 5 and 15 min) were determined. After that, those independent variables were randomly matched as 100 MPa–50 °C–15 min, 300 MPa–70 °C–5 min and 500 MPa–90 °C–1 min in order to carry out experimental study. The prediction accuracy of the models was determined in terms of error value related with actual and predicted outputs for the same process conditions. The error results for obtained models were given in Table 3. The results showed that the relative error values for all responses at different high pressure processing conditions are lower than 10% which means acceptable error value in modelling study [3, 29]. Thus, it can be concluded that the obtained models can be used to predict numerically the effect of high pressure processing conditions on the critical quality attributes of black carrot pomace extracts.

Table 4 Optimization study criteria for independent and dependent variables

Variables	Goal/limitation
Independent	
Pressure (MPa)	In range ($0.1 \le P \le 600$)
Temperature (°C)	In range ($25 \le T \le 90$)
Time (min)	In range ($0 \le t \le 20$)
Dependent	
TMA (mg/L)	Maximize
TPC (mg/L)	Maximize
AA (%)	Maximize
ΔE*	In range ($2 < \Delta E^* < 10$)
PCR (%)	Minimize ($PCR < 25$)

Optimization

In order to optimize bioactive compounds extraction conditions from black carrot pomace with assistance of high pressure processing, the developed mathematical models were used. The determined goals and limitations for optimization study were given in Table 4. The independent variables were defined in range with certain intervals, numerically, $0.1 \le P \le 600$ MPa for pressure, $25 \le T \le 90$ °C for temperature and $0 \le t \le 20$ min for processing time. In the presented study, some of the dependent responses, such as; TMA, TPC and AA yields were aimed to maximize in the black carrot extracts. During the application of experimental runs, it was recognized that the pectin molecules which are high in the black carrot tissue started to gelatinize remarkably when the process conditions were higher than $P > 400$ MPa and

Table 3 The validation study results for different high pressure processing conditions

Dependents	Independents			Responses		Error[a] (%)
	P (MPa)	*T* (°C)	*t* (min)	Predicted	Actual	
TMA (mg/L)	100	50	15	314.45	308.06 ± 6.16	2.14 ± 1.80
	300	70	5	322.05	326.58 ± 7.19	2.27 ± 0.86
	500	90	1	343.36	335.33 ± 5.66	2.45 ± 1.65
TF (mg/L)	100	50	15	101.80	99.93 ± 1.20	1.84 ± 1.18
	300	70	5	106.24	105.49 ± 2.17	1.89 ± 0.32
	500	90	1	111.45	109.85 ± 2.51	1.80 ± 1.15
AA (%)	100	50	15	89.14	91.22 ± 0.44	2.34 ± 0.49
	300	70	5	88.84	91.49 ± 0.39	2.98 ± 0.43
	500	90	1	84.70	87.88 ± 0.28	3.81 ± 0.33
ΔE*	100	50	15	5.33	5.51 ± 0.02	3.34 ± 0.32
	300	70	5	5.78	5.88 ± 0.03	1.59 ± 0.59
	500	90	1	15.51	14.88 ± 0.09	4.04 ± 0.59
PCR (%)	100	50	15	12.15	11.55 ± 0.24	4.90 ± 1.98
	300	70	5	18.40	18.53 ± 0.20	1.13 ± 0.24
	500	90	1	26.85	27.77 ± 0.85	3.98 ± 2.18

[a]Relative error values were calculated according to experimental and predicted outputs

Table 5 The best solutions for defined limitations and goals of optimization study

Solution number	Pressure (*P*, MPa)	Temperature (*T*, °C)	Time (*t*, min)	TMA (mg/L)	TPC (mg/L)	AA (%)	ΔE*	PCR (%)	Desirability
1	266.6	78.3	13.6	105.7	333.9	89.3	7.7	21.6	0.333
2	268.9	77.6	13.9	105.4	333.8	89.4	7.6	21.8	0.323
3	284.0	80.5	10.9	107.0	334.3	88.6	8.5	21.5	0.319
4	265.4	76.2	13.1	105.5	332.0	89.3	7.4	21.0	0.309
5	268.4	78.2	13.5	105.7	333.9	89.3	7.7	21.7	0.302

¤*TMA* is total monomeric anthocyanin, *TPC* is total phenolic content, *AA* is antioxidant activity, *ΔE** is total color difference and PCR is polymeric color ratio

$T > 55$ °C (data not shown). This is undesirable for extraction technology while desirable for jam and jelly industry. The pectin gels are able to limit mass transfer, which results with decreasing of the extraction yield of the target bioactive compounds. In order to eliminate pectin gelatinization, some limitations must be taken into consideration. It was observed that ΔE* and *PCR* values are suitable as dependent parameters to achieve that aim. Therefore, while ΔE* was defined as $2 < \Delta E^* < 10$, *PCR* value was aimed to be minimum and lower than 25 ($PCR < 25$) for the optimization study. Thus, the high pressure processing conditions causing to pectin molecules gelatinization can be eliminated.

According to the defined limitations and goals, the best five solutions were listed in Table 5. The highest desirability value was calculated as 0.333 at 266.6 MPa, 78.3 °C and 13.6 min for high pressure processing conditions. It means that if those process conditions were applied to the black carrot pomace, a black carrot pomace extract which contents the highest bioactive compounds with the best quality attributes would be obtained.

Discussion

High pressure assisted extraction mechanism can be explained with three different processing stages called pressure boost, maintaining and relief stages [11]. At the first stage, the pressure is gradually increased from the atmospheric pressure to the processing pressure in a short period of time. Thus, the disruption of the plant tissue, cellular wall, membrane and organelles contenting valuable nutrients can be occurred and, moreover, the mass transfer of the extraction solvents into the materials and solution of the valuable nutrients can be enhanced. This phenomenon is explained by mass transfer theory defining proportional relationship between the mass transfer rate and pressure/resistance of mass transfer. Simply, the theory is based on higher matter solution at higher pressure conditions. In the light of this information, the pressure difference between the medium and the animal/plant intracellular is main force for mass transfer of bioactive compounds from the high density to the low. At the second stage, because of the first stage situations, the extraction solvent diffuses into the cells rapidly and interacts with the target intracellular materials. The second stage is important for increasing the yield of bioactive compounds. This can be achieved by extending holding time of high pressure. At the last stage, the cell pressure quickly decreases from the processing pressure to the atmospheric pressure, which causes dramatic changing in hydrogen bonds, ionic bonds, and hydrophobic interactions that control the polymeric structure of the cells. Thus, the cells are able to expand and that expansion is responsible from porous, broken or loose in the cellular structures, which are results with improving of mass diffusion of bioactive compounds [10, 11].

Corrales et al. [18] also reported that the highest anthocyanin compounds yield was obtained for the grape skin extracts processed at 200 MPa, constant temperature (70 °C) and time (30 min). They also tried different processing time between 30 and 90 min at constant pressure (600 MPa) and temperature (70 °C) and the best processing time was 30 min for collecting extracts with high anthocyanin compounds. In addition, they showed that the yield of anthocyanins were higher for the extracts processed at mild temperature than samples processed at lowest (20 °C) or highest (90 °C) temperature. As a result, according to the findings of the study, the extracts treated with 200 MPa, 50–70 °C and 30 min would have higher yields of anthocyanin compounds. Similarly, in the presented study, there is tendency for obtaining higher anthocyanin yields at the conditions of 0.1–300 MPa pressure, 70–90 °C temperature and 0.1–10 min processing time. In addition, Butz et al. [30] reported that 100 MPa high pressure processing is sufficient to disrupt the cell wall and vacuoles, thus increasing mass transfer of valuable components through the cell to the medium. In an optimization study, Fernandes et al. [20] researched the effect of different pressures (0–500 MPa), processing times (5–15 min) and solvent concentrations on recovery of anthocyanin compounds from pansies at room temperature. The study revealed higher yield of anthocyanins up to 200 MPa

which stabilized beyond the pressure threshold. Finally, they calculated 384 MPa and 15 min is best processing conditions for maximizing antioxidant activity, total monomeric anthocyanins and flavonoids in pansies based extract. In our optimization study, the optimum high pressure processing conditions for extraction of bioactive compounds from black carrot pomace was calculated as 266.6 MPa, 78.3 °C and 13.6 min. In our case, the optimum pressure value is lesser than optimum pressure value of Fernandes et al. [20] study. The possible explanation is different extraction temperature which is remarkably higher in our study.

Anthocyanin compounds have a significant benefit on human health, therefore, minimal loss during processing must be considered. The stability of anthocyanin compounds is depended on the medium conditions such as pH, temperature, enzyme, oxygen, light, concentration, copigments and metallic ions [31]. Thermal processing triggers anthocyanins degradation reactions resulting with the formation of polymers, dominating brown color in the medium [32]. However, in our case, the lowest monomeric anthocyanin contents were achieved through high pressure treated samples. In addition, the TMA model predicted a linear decreasing tendency even at low temperature, when processing temperature and time were fixed (Fig. 1). Moreover, enzymatic degradation of anthocyanins for black carrot pomace samples does not explain the decreasing of monomeric anthocyanins in the samples, because the mash of black carrots expose to thermal processing for inactivation of enzymes during juice processing line. To explain the degradation of anthocyanins under high pressure, among all the factors, pH is the most suitable one. The acid dissociation constant depends on the pressure and temperature for weak acids and is mathematically described by Plank's equation $\left(\frac{\delta \ln K_a}{\delta P} = -\frac{\Delta V^o}{RT}\right)$. In a weak acid medium, ΔV^o values could be negatively high, resulting with large decreases in pH under extreme pressure (0.4 pH unit/100 MPa) [33]. One of the recent studies showed that pH is one of the main factors affecting ability of anthocyanin compounds to resist degradation at extreme conditions [34]. In their study, they treated purple sweet potato extracts at 90 °C in different pH medium ranging between 3 and 7. Their findings highlighted that pH medium affects stability of anthocyanin compounds. Moreover, in the pH = 3 medium, the monomeric anthocyanins changed into new polymers during thermal treatment. They also showed that anthocyanin degradation resulted with increasing polymeric color because of melanoidins formations and condensation reactions. This mechanism is also good tool to explain dramatic change in color values (ΔE*) and polymeric color ratio (*PCR*) in the presented study extracts (Figs. 4, 5).

Jun et al. [35] processed green tea leaves with different high pressure and processing time, in order to enhance the extraction yields of polyphenols. They showed that when the pressure increased from 100 to 600 MPa, the extraction yields of green tea polyphenols increased from 15 to 30%. However, they reported that there is no difference for processing time ranged from 1 to 10 min at 500 MPa and 1 min is enough for the extraction of green tea polyphenols. Similar result was reported by Raj et al. [36] for gooseberry juice processed with 200–500 MPa, 30–60 °C, 1 s–20 min. They determined that phenolic content increased to almost 20% after the HPP processing at 500 MPa/50 °C/20 min. They explained that increase with pressurization effect on enhancing the extractability and solubility of the phenolic compounds. Yuan et al. [37] treated aronia berry puree with HPP conditions (200–600 MPa, 2.5–5 min and ≤ 33 °C), and the results indicated that the highest phenolic contents and antioxidant capacities were obtained at 400 MPa for 5 min. Similarly, the effects high pressure, temperature and processing time on yields of black grape skin anthocyanins and antioxidant activity were also studied by Corrales et al. [18]. The results showed that the antioxidant activity is increased up to three times higher than untreated sample, when the pressure is applied at 200, 400 and 600 MPa at constant 20 °C temperature and 30 min processing time. Moreover, at constant processing pressure (600 MPa) and temperature (70 °C), the antioxidant yield of black grape skin extracts is increased for higher processing time. The lower antioxidant yield was determined for extracts treated with high pressure processing at 90 °C than those treated at 70 °C. However, although the antioxidant yield was not changed importantly for different applied high pressures, the highest yields were obtained for 400 and 600 MPa high pressures at constant temperature and time. In the presented study, similarly, the models showed that the antioxidant activity would be higher for the processing conditions that are ranged between 350 and 600 MPa, 40–75 °C and 15–20 min.

For presented study, a surprising result was observed for pectin molecules in the black carrot pomace extract, which started to gelatinize under high pressure and moderate temperature conditions ($P > 400$ MPa and $T > 55$ °C). Similar results were demonstrated by Bauer et al. [38] for starch molecules. They determined that starch gelatinization was highly sensitive to changes in temperature, pressure and treatment time. In their study, especially at 57 °C temperature and higher than 200 MPa pressure, the wheat starch was completely gelatinized within 15 min holding time. Abe et al. [39] reported that the combination of heat and high pressure treatments resulted as complete gelatinization in sweet potato starch. Moreover, according to microscopic observations of pressurized wheat starch, Douzals et al. [40] reported that starch gelatinization began above a pressure of 300 MPa without previous heating. As a result, gelatinization of polysaccharides such as pectin and starch is a big

disadvantage in extraction of bioactive compounds due to causing remarkable mass transfer resistance, but it can be exciting result from the perspective of jelly industry. Therefore, the potential contribution of HPP to the jelly industry must be put forth with the further studies.

Conclusion

The bioactive compounds of the black carrot pomace were successfully extracted by assistance of high pressure processing (HPP) for various experimental conditions including pressure, temperature and processing time. The results collected from experimental conditions were mathematically modelled with high correlation ($R^2 > 0.94$) for total monomeric anthocyanin contents (TMA), total phenolic compounds (TMA), antioxidant activity, polymeric color ratio (PCR), and total color difference (ΔE*) by using of response surface methodology. It is obvious that high pressure is useful tool for improving the extraction yields of bioactive compounds from black carrot pomace. The optimization study showed that when a high pressure processing at 266.6 MPa, 78.3 °C and 13.6 min was applied on black carrot pomace, the obtained extracts would have the highest yield of bioactive compounds as well as meeting with adequate quality attributes.

In a previous research study, we tried also to optimize thermosonic extraction conditions (ultrasound energy density and temperature) of anthocyanin compounds from the same material used in the presented study. The average yield of anthocyanin compounds was calculated to be 27.6 ± 7.7% at the optimum thermosonic processing conditions for the five cyanidin based anthocyanin compounds in the black carrot pomace [3]. In the presented study, if the optimum HPP conditions (266.6 MPa, 78.3 °C and 13.6 min) were applied to the pomace, the yield of TMA, TPC and AA in the obtained extract would be as 55.5, 51.4 and 18.9% when compared to the untreated samples; respectively. In addition, the average yield of bioactive compounds was calculated to be 41.9 ± 20.0% at the optimum HPP conditions. These results demonstrated that the extraction yield of the bioactive compounds from black carrot pomace would be remarkably higher with assistance of appropriate HPP conditions than assistance of ultrasound processing conditions.

In conclusion, HPP is promising technology to extract bioactive compounds. Further, this green technology is able to reduce extraction time, solvent consumption, bioactive compounds degradation, and environmental pollution caused by toxic solvents. Therefore, in the near future, HPP technology has a high potential to be used as assistant in bioactive compounds extraction from plant/animal sources.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12649-021-01431-z.

Acknowledgements This study was supported by the Scientific and Technological Research Council of Turkey-Science Fellowships and Grant Programmes Department (TUBITAK-BIDEP) with grant of 2214-International Doctoral Research Fellowship Programme. Author Agcam would like to thank the TUBITAK-BIDEP for grant supports (Grant No: 1059B141400558). The study was also supported by Cukurova University (Project No: ZF2014D1 and ZF2014BAP1), so the authors thank to for its financial supports. Authors are grateful to the Ohio State University-High Pressure Processing (HPP) Laboratory for supporting about access to the HPP equipment. Finally, the authors thank to TARGID Juicing Company (Turkey) for providing the black carrots and pomace during study.

References

1. The History of Carrots. (2018) http://www.carrotmuseum.co.uk/history.html. Accessed 22 Nov 2018
2. TUİK. (2018). http://www.turkstat.gov.tr/UstMenu.do?metod=temelist. Accessed 22 Nov 2018
3. Agcam, E., Akyildiz, A., Balasubramaniam, V.M.: Optimization of anthocyanins extraction from black carrot pomace with thermosonication. Food Chem. **237**, 461–470 (2017). https://doi.org/10.1016/j.foodchem.2017.05.098
4. Kamiloglu, S., Ozkan, G., Isik, H., Horoz, O., Van Camp, J., Capanoglu, E.: Black carrot pomace as a source of polyphenols for enhancing the nutritional value of cake: an in vitro digestion study with a standardized static model. Lwt-Food Sci. Technol. **77**, 475–481 (2017)
5. Yilmaz, F.M., Bilek, S.E.: Ultrasound-assisted vacuum impregnation on the fortification of fresh-cut apple with calcium and black carrot phenolics. Ultrason. Sonochem. **48**, 509–516 (2018)
6. Kirca, A., Ozkan, M., Cemeroglu, B.: Stability of black carrot anthocyanins in various fruit juices and nectars. Food Chem. **97**(4), 598–605 (2006). https://doi.org/10.1016/j.foodchem.2005.05.036
7. Kirca, A., Ozkan, M., Cemeroglu, B.: Storage stability of strawberry jam color enhanced with black carrot juice concentrate. J. Food Process Pres. **31**(5), 531–545 (2007). https://doi.org/10.1111/j.1745-4549.2007.00140.x
8. Koç, B.E., Türkyılmaz, M., Özkan, M.: Use of black carrot juice concentrate as colorant in hard candies and storage stability of monomeric anthocyanins. Acad. Food J. **10**(1), 30–39 (2012)
9. Puertolas, E., Cregenzan, O., Luengo, E., Alvarez, I., Raso, J.: Pulsed-electric-field-assisted extraction of anthocyanins from purple-fleshed potato. Food Chem. **136**(3–4), 1330–1336 (2013). https://doi.org/10.1016/j.foodchem.2012.09.080
10. Zhang, S.Q., Zhu, J.J., Wang, C.Z.: Novel high pressure extraction technology. Int. J. Pharm. **278**(2), 471–474 (2004). https://doi.org/10.1016/j.ijpharm.2004.02.029
11. Huang, H.W., Hsu, C.P., Yang, B.B., Wang, C.Y.: Advances in the extraction of natural ingredients by high pressure extraction technology. Trends Food Sci. Technol. **33**(1), 54–62 (2013). https://doi.org/10.1016/j.tifs.2013.07.001
12. Rastogi, N.K., Nguyen, L.T., Jiang, B., Balasubramaniam, V.M.: Improvement in texture of pressure-assisted thermally processed carrots by combined pretreatment using response surface methodology. Food Bioprocess Tech. **3**(5), 762–771 (2010)
13. Prasad, K.N., Yang, B., Zhao, M.M., Wei, X.Y., Jiang, Y.M., Chen, F.: High pressure extraction of corilagin from longan

(*Dimocarpus longan* Lour.) fruit pericarp. Sep. Purif. Technol. **70**(1), 41–45 (2009). https://doi.org/10.1016/j.seppur.2009.08.009
14. Prasad, K.N., Yang, B., Zhao, M.M., Wang, B.S., Chen, F., Jiang, Y.M.: Effects of high-pressure treatment on the extraction yield, phenolic content and antioxidant activity of litchi (*Litchi chinensis* Sonn.) fruit pericarp. Int. J. Food Sci. Tech. **44**(5), 960–966 (2009). https://doi.org/10.1111/j.1365-2621.2008.01768.x
15. Prasad, K.N., Yang, B., Zhao, M., Sun, J., Wei, X., Jiang, Y.: Effects of high pressure or ultrasonic treatment on extraction yield and antioxidant activity of pericarp tissues of longan fruit. J. Food Biochem. **34**, 838–855 (2010). https://doi.org/10.1111/j.1745-4514.2010.00335.x
16. Lee, A.R., Choi, S.-H., Choi, H.-W., Ko, J.-H., Kim, W., Kim, D.-O., Kim, B.-Y., Baik, M.-Y.: Optimization of ultra high pressure extraction (UHPE) condition for puffed ginseng using response surface methodology. Food Sci. Biotechnol. **23**(4), 1151–1157 (2014). https://doi.org/10.1007/s10068-014-0157-6
17. Briones-Labarca, V., Plaza-Morales, M., Giovagnoli-Vicuna, C., Jamett, F.: High hydrostatic pressure and ultrasound extractions of antioxidant compounds, sulforaphane and fatty acids from Chilean papaya (*Vasconcellea pubescens*) seeds: effects of extraction conditions and methods. Lwt-Food Sci. Technol. **60**(1), 525–534 (2015). https://doi.org/10.1016/j.lwt.2014.07.057
18. Corrales, M., García, A.F., Butz, P., Tauscher, B.: Extraction of anthocyanins from grape skins assisted by high hydrostatic pressure. J. Food Eng. **90**(4), 415–421 (2009). https://doi.org/10.1016/j.jfoodeng.2008.07.003
19. Prasad, K.N., Yang, E., Yi, C., Zhao, M., Jiang, Y.: Effects of high pressure extraction on the extraction yield, total phenolic content and antioxidant activity of longan fruit pericarp. Innov. Food Sci. Emerg. **10**(2), 155–159 (2009). https://doi.org/10.1016/j.ifset.2008.11.007
20. Fernandes, L., Casal, S.I.P., Pereira, J.A., Ramalhosa, E., Saraiva, J.A.: Optimization of high pressure bioactive compounds extraction from pansies (*Viola* x *wittrockiana*) by response surface methodology. High Pressure Res. **37**(3), 415–429 (2017)
21. Prasad, K.N., Yang, B., Zhao, M.M., Ruenroengklin, N., Jiang, Y.M.: Application of ultrasonication or high-pressure extraction of flavonoids from litchi fruit pericarp. J. Food Process Eng. **32**(6), 828–843 (2009). https://doi.org/10.1111/j.1745-4530.2008.00247.x
22. Zhang, S.Q., Chen, R.Z., Wang, C.Z.: Experiment study on ultra-high pressure extraction of ginsenosides. J. Food Eng.. **79**(1), 1–5 (2007)
23. Agcam, E., Akyıldız, A.: Effects of different solvents and acid concentrations on extraction of anthocyanins from black carrot pomace. J. Food **40**(3), 149–156 (2015). https://doi.org/10.15237/gida.GD14064
24. Dhakal, S., Balasubramaniam, V.M., Cocuron, J.C., Alonso, A.P., Agcam, E., Kamat, S.: Pressure-thermal kinetics of furan formation in selected fruit and vegetable juices. Food Bioprocess Tech. **10**(11), 1959–1969 (2017)
25. Abdullakasim, P., Songchitsomboon, S., Techagumpuch, M., Balee, N., Swatsitang, P., Sungpuag, P.: Antioxidant capacity, total phenolics and sugar content of selected Thai health beverages. Int. J. Food Sci. Nutr. **58**(1), 77–85 (2007). https://doi.org/10.1080/09637480601140946
26. Giusti, M.M., Wrolstad, R.E.: Characterization and measurement of anthocyanins by UV-visible spectroscopy. In: Wrolstad, R.E., Schwartz, S.J. (eds.) Current protocols in food analytical chemistry, pp. 683–695. Wiley, New York (2001)
27. Klimczak, I., Małecka, M., Szlachta, M., Gliszczyńska-Świgło, A.: Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. J. Food Compos. Anal. **20**(3–4), 313–322 (2007). https://doi.org/10.1016/j.jfca.2006.02.012
28. Illera, A.E., Sanz, M.T., Benito-Román, O., Varona, S., Beltrán, S., Melgosa, R., Solaesa, A.G.: Effect of thermosonication batch treatment on enzyme inactivation kinetics and other quality parameters of cloudy apple juice. Innov. Food Sci. Emerg. **47**, 71–80 (2018). https://doi.org/10.1016/j.ifset.2018.02.001
29. Bimakr, M., Rahman, R.A., Taip, F.S., Adzahan, N.M., Sarker, M.Z., Ganjloo, A.: Supercritical carbon dioxide extraction of seed oil from winter melon (*Benincasa hispida*) and its antioxidant activity and fatty acid composition. Molecules **18**(1), 997–1014 (2013). https://doi.org/10.3390/molecules18010997
30. Butz, P., Koller, W.D., Tauscher, B., Wolf, S.: Ultra-high pressure processing of onions: chemical and sensory changes. Food. Sci. Technol-Leb **27**(5), 463–467 (1994)
31. Martynenko, A., Chen, Y.: Degradation kinetics of total anthocyanins and formation of polymeric color in blueberry hydrothermodynamic (HTD) processing. J. Food Eng. **171**, 44–51 (2016). https://doi.org/10.1016/j.jfoodeng.2015.10.008
32. Sinela, A., Rawat, N., Mertz, C., Achir, N., Fulcrand, H., Dornier, M.: Anthocyanins degradation during storage of *Hibiscus sabdariffa* extract and evolution of its degradation products. Food Chem. **214**, 234–241 (2017). https://doi.org/10.1016/j.foodchem.2016.07.071
33. Gayán, E., Condón, S., Álvarez, I., Nabakabaya, M., Mackey, B.: Effect of pressure-induced changes in the ionization equilibria of buffers on inactivation of *Escherichia coli* and *Staphylococcus aureus* by high hydrostatic pressure. Appl. Environ. Microb. **79**, 4041–4047 (2013)
34. Jiang, T., Mao, Y., Sui, L., Yang, N., Li, S., Zhu, Z., Wang, C., Yin, S., He, J., He, Y.: Degradation of anthocyanins and polymeric color formation during heat treatment of purple sweet potato extract at different pH. Food Chem. **274**, 460–470 (2019). https://doi.org/10.1016/j.foodchem.2018.07.141
35. Jun, X., Deji, S., Shou, Z., Bingbing, L., Ye, L., Rui, Z.: Characterization of polyphenols from green tea leaves using a high hydrostatic pressure extraction. Int. J. Pharm. **382**(1–2), 139–143 (2009). https://doi.org/10.1016/j.ijpharm.2009.08.023
36. Raj, A.S., Chakraborty, S., Rao, P.S.: Thermal assisted high-pressure processing of Indian gooseberry (*Embilica officinalis* L.) juice: impact on colour and nutritional attributes. Lwt **99**, 119–127 (2019). https://doi.org/10.1016/j.lwt.2018.09.051
37. Yuan, B., Danao, M.-G.C., Stratton, J.E., Weier, S.A., Weller, C.L., Lu, M.: High pressure processing (HPP) of aronia berry purée: effects on physicochemical properties, microbial counts, bioactive compounds, and antioxidant capacities. Innov. Food Sci. Emerg. **47**, 249–255 (2018). https://doi.org/10.1016/j.ifset.2018.03.009
38. Bauer, B.A., Knorr, D.: The impact of pressure, temperature and treatment time on starches: pressure-induced starch gelatinisation as pressure time temperature indicator for high hydrostatic pressure processing. J. Food Eng. **68**(3), 329–334 (2005). https://doi.org/10.1016/j.jfoodeng.2004.06.007
39. Abe, S., Takimoto, S., Yamamuro, Y., Tau, K., Takenaga, F., Suzuki, K., Oda, M.: High-pressure and heat pretreatment effects on rehydration and quality of sweet potato. Am. J. Food Technol. **6**(1), 63–71 (2011). https://doi.org/10.3923/ajft.2011.63.71
40. Douzals, J.P., Marechal, P.A., Coquille, J.C., Gervais, P.: Microscopic study of starch gelatinization under high hydrostatic pressure. J. Agric. Food Chem. **44**(6), 1403–1408 (1996)

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Authors and Affiliations

E. Agcam[1] · A. Akyıldız[1] · S. Kamat[2] · V. M. Balasubramaniam[2,3]

[1] Department of Food Engineering, Faculty of Agriculture, Cukurova University, Adana, Turkey

[2] Department of Food Science and Technology, The Ohio State University, Columbus, OH, USA

[3] Department of Food, Agricultural and Biological Engineering, The Ohio State University, Columbus, OH, USA