INNSPUB

International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 16, No. 4, p. 1-10, 2020

RESEARCH PAPER OPEN ACCESS

Effect of thermal treatment on quality of black cherry tomatoes (*Solanum lycopersicum* cv. OG): optimization of the blanching parameters

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Article published on April 19, 2020

Key words: Black cherry tomatoes, Optimization, Blanching, Peroxidase, Bioactive compounds

Abstract

The objective of this study was to identify the optimal parameters of blanching to inactivate enzymes and convert insoluble protopectin into soluble pectin while maintaining maximum levels of bioactive compounds in black cherry tomatoes (*Solanum lycopersicum* cv. OG) vacuum-infiltrated. Blanching conditions for black cherry tomatoes were optimized by using Response Surface Methodology (RSM) with blanching temperature (83-97°C) and time (69-111s). Before blanching, fruits were immersed in the water under a vacuum level of 620mmHg and treatment time of 22 min to improve heat transfer capacity and thus limit thermal damages in the product due to the replacement of gases inside the fruit spores by the liquid. The optimal conditions for the blanching process selected by Central Composite Design (CCD) were 89°C for 80s. With these conditions, the lowest value of remaining peroxidase activity and the highest contents of pectin (2.35%) and bioactive compounds (anthocyanin 4.23mgCE/100g, lycopene 44.93µg/g, vitamin C 48.39mg/100g, total phenolic 43.14mgGAE/100g) in tomatoes were observed.

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Introduction

'Black" or 'purple' cherry tomatoes are subspecies of *Solanum lycopersicum* (Zhang *et al.*, 2018) and exhibit a dirty purplish-brown colour on their skin (Mes *et al.*, 2008). They are rich in bioactive compounds (lycopene 54.20 ± 0.94mg/100g dried-weight, β-Carotene 3.75 ± 0.07mg/100g dried-weight) (Choi *et al.*, 2014). Lycopene is the most abundant (about 80-90%) and the highest antioxidant natural carotenoid found in tomatoes (Alda *et al.*, 2009). Epidemiological studies have shown that this red pigment has the potential to reduce the risk of chronic diseases, most notably prostate cancer (Ford and Erdman, 2012). Lycopene also plays an important role in the prevention of cardiovascular disease (Mordente *et al.*, 2011). Min J.Y and Min K.B. (2014) found that consuming a large quantity of lycopene-abundant foods led to a lower risk of mortality from Alzheimer's disease in adults. Kaur *et al.* (2011) also indicated that lycopene had a protective ability against oxidative stress, which is beneficial in treating Parkinson's disease and other neurological abnormalities.

In addition to carotenoids, tomatoes are good for other antioxidant compounds such as vitamin C and phenolics which also inhibit reactive oxygen species causing many dangerous diseases (Ilahy *et al.*, 2009). It has been noticed that the content of phenolic compounds and carotenoid pigments, particularly lycopene in black cherry tomatoes are higher than some red tomato varieties (Zhang *et al.*, 2018). Especially, black cherry tomatoes also can produce a phytochemical of anthocyanin predominantly in the skin (Li *et al.*, 2011). Anthocyanin has been proven to be associated with many health benefits, reduces cancer cell proliferation, protects against cardiovascular disease, prevents obesity and diabetes (Lila, 2004).

Because of their health benefits, black cherry tomatoes can be used as fresh vegetables or processed into popular liquid products such as puree, ketchup and sauce (Zhang *et al.*, 2018). Blanching of raw material is a necessary stage in the processing of these products to inactivate enzymes and destroy microorganisms that cause undesirable organoleptic and nutritional changes thereafter (Xiao *et al.*, 2014). The conversion from protopectin into soluble pectin also took place under the effect of high temperature during blanching (Ramos-Aguilar *et al.*, 2015). This pectin ingredient presenting in tomato sauces and similar products helps to create a characteristic viscous texture (Mesbahi *et al.*, 2005). However, like other thermal treatment processes, blanching can lead to a significant loss of heat-sensitive nutritional components.

Therefore, the aim of the present work was an optimization of the blanching thermal treatment parameters of black cherry tomatoes to inactivate the peroxidase and convert protopectin to soluble pectin as much as possible but prevent the destruction of bioactive compounds caused by heat, especially anthocyanin in the outer skin of fruits.

Materials and methods

Tomato fruits

Black cherry tomato (cv. OG) seeds were provided by the F1508 seed store (Ho Chi Minh City, Vietnam) and grown at Nam Long farm, Vinh Long province, Vietnam. Tomatoes were harvested at full ripeness (32 days after fruit formation). All fruits with diseases and defects were removed. Fruits were packed into perforated PVC and then cardboard boxes. They were transported to the Food Technology Laboratory of Can Tho University, Vietnam within 1 hr. Tomatoes were washed and dipped into the water which was aerated with ozone for 15 min by a 2-nozzle ozone generator (Z755, Vietnam, ozone-generating of 80.4mg/h, the sample weight of 1500g, the ratio of fruits and water was 1:2). The infiltration process was then carried out in the vacuum equipment (Japan). The vacuum level and treatment time were chosen as 620mmHg and 22 min, respectively and a ratio of material and distilled water was 1:1. After vacuum infiltration, the mixture was brought to the atmospheric condition and kept for another 15 min. Fruits were drained and used for the blanching experiment.

Experimental design

The experiment was designed by the Portable Statgraphics Centurion software (version 15.2.11.0, U.S.A.) with two factors of blanching temperature

(X_1) and time (X_2). The Response Surface Methodology (RSM) with a model of Central Composite Design (CCD) was applied. Before designing the optimization experiment, a preliminary investigation was carried out for a wide range of blanching temperatures (75-95°C) and blanching time (30-150s) and as a result, the narrower study ranges were chosen as 83-97°C and 69-111s. The actual and coded values of each parameter were presented in Table 1. Each factor was encoded with five levels: -1.414, -1 (low), 0 (central), +1 (high) and +1,414. Total samples were 14, including six replications of the central point. The blanching process was carried out in a thermostatic bath (Rex C-90, Memmert, Germany) with the ratio of material and distilled water was 1:2. After that, fruits were cooled quickly by tap water. The remaining peroxidase activity and content of pectin and bioactive compounds of samples after blanching were used as indicators.

Table 1. Actual and coded values of the variables.

Run	Blanching temperature	Blanching time
1	85 (-1)	105 (+1)
2	90 (0)	90 (0)
3	90 (0)	90 (0)
4	90 (0)	69 (-1.414)
5	85 (-1)	75 (-1)
6	83 (-1.414)	90 (0)
7	90 (0)	111 (+1.414)
8	90 (0)	90 (0)
9	97 (+1.414)	90 (0)
10	90 (0)	90 (0)
11	95 (+1)	75 (-1)
12	90 (0)	90 (0)
13	90 (0)	90 (0)
14	95 (+1)	105 (+1)

Notes: Units of blanching temperature and time were °C and s, respectively; Numbers in parentheses were coded values

Analytical methods

Peroxidase (POD) activity

The POD activity was determined by the colourimetric method based on reaction with guaiacol (Goncalves *et al.*, 2007; Morales-Blancas *et al.*, 2002) with some modifications. Tomato puree (20g) was extracted with 100mL of 1 M NaCl solution for 10 min on a shaker (SK600, Lab Companion, Korea) at a speed of 180rpm. The mixture was then separated by a centrifuge (Z323K, Hermle Labortechnik GmbH, Germany) at 7000×g for 10 min. The substrate solution was prepared daily by mixing 0.1mL guaiacol (99.5%), 0.1mL H_2O_2 (30%) and 99.8mL potassium phosphate buffer (0.1 M). The supernatant (0.12mL) was mixed with 3.48mL of substrate solution and the absorbance increase was recorded after 20 min at 470nm by a Spectrophotometer UV-VIS (722N, Inesa, China) using a blank prepared with 0.12mL distilled water and 3.48mL POD substrate solution. The remaining enzyme activity was calculated by equation 1. Where U_o and U_t were enzyme activity at the beginning and after blanching (%), respectively.

$$\text{Peroxidase (\%)} = \frac{U_t}{U_o} \times 100 \qquad (1)$$

Pectin content

The pectin content was determined by the pectate calcium method (Mai, 2005). Tomato puree (50g) was filled to a volume of 100mL with distilled water and filtered through a filter paper. The filtrate (20mL) was mixed with 100mL of 0.1N NaOH solution. The saponification of pectin was carried out for 7 hrs. The mixture was added 50mL of 0.1N CH_3COOH solution, left for 5 min and then added 50mL of 1 N $CaCl_2$ solution. After 1 hr, the sample was boiled for 5 min and then filtered through a filter paper. The filter paper containing the precipitate was dried at 105°C to a constant weight. The pectin content was calculated using equation 2. Where *P* is the precipitate weight (g), 0.92 is the conversion coefficient from calcium pectate to pectin, *m* is the sample weight (g).

$$\text{Pectin (\%)} = \frac{P \times 0.92}{20} \times \frac{100}{m} \times 100 \qquad (2)$$

Anthocyanin content

The anthocyanin content was determined by the pH differential method (Lee *et al.*, 2005) with some modifications. Tomato puree (5g) was filled to a volume of 50mL with ethanol/water (1/1) solvent containing 1% HCl and extracted for 60 min. The mixture was then separated by a centrifuge at 7000×g for 10 min. The supernatant was diluted with two buffers of pH 1.0 and 4.5 and read the absorbance at both 520 and 700nm versus a blank of distilled water. The anthocyanin content was expressed as cyanidin-3-glucoside equivalent (CE) and calculated

by equation 3. Where *A* is (A_{520nm}-A_{700nm})pH 1.0 - (A_{520nm}-A_{700nm})pH 4.5, *M* is 449.2g/mol for cyanidin-3-glucoside, *k* is the dilution factor, *l* is the pathlength (cm), *ε* is 26900 ($L \times mol^{-1} \times cm^{-1}$) - molar extinction coefficient for cyanidin-3-glucoside, *V* is the extract volume (mL), *m* is the sample weight (g).

$$\text{Anthocyanin (mgCE/100g)} = \frac{A \times M \times k \times V}{m \times \varepsilon \times l} \times 100 \times 1000 \quad (3)$$

Lycopene content

The lycopene content was determined by the low volume hexane extraction method (Davis *et al.*, 2003; Fish *et al.*, 2002). Tomato puree (0.6g) was mixed with 5mL of acetone containing 0.05% butylated hydroxytoluene, 5mL of 95% ethanol, 10mL of hexane and extracted for 15 min on a shaker at a speed of 180rpm. The mixture was then added 3mL of deionized water and shook for another 5 min. The vial was left for 5 min. The absorbance of the supernatant layer was read at 503nm against a blank of hexane. The lycopene content was determined using equation 4. Where A_{503} is the absorbance of the extract at 503nm, *m* is the sample weight (g).

$$\text{Lycopene (µg/g)} = \frac{A_{503} \times 31.2}{m} \quad (4)$$

Vitamin C content

The vitamin C content was determined by the titration method (Lam *et al.*, 2004). Tomato puree (10g) was filled to a volume of 100mL with 5% HCl solution and filtered through a filter paper. The filtrate (10mL) was added 5 drops of the 1% starch solution and titrated with the 0.001N KIO_3/KI solution until the blue-black colour appears. For the control, the sample extract was replaced by the 1% HCl solution. The vitamin C content was calculated by equation 5. Where *a* and *b* are the volume of 0.001N KIO_3/KI solution used for titration the extract and the control, respectively (mL), *100* is the extract volume (mL), *0.088* is the weight of ascorbic acid corresponds to 1mL of 0.001 N KIO_3/KI solution (mg), *m* is the sample weight (g).

$$\text{Vitamin C (mg/100g)} = \frac{(a-b) \times 0.088 \times 100}{10} \times \frac{100}{m} \quad (5)$$

Total phenolic content

The total phenolic content was determined using Folin-Ciocalteu reagent (Teixeira *et al.*, 2013) with some modifications. Tomato puree (5g) was filled to a volume of 50mL with 95% ethanol and extracted for 60 min. The mixture was then separated by a centrifuge at 7000×g for 10 min. The supernatant (0.2mL) was added 1.0mL of 10% Folin-Ciocalteu reagent, left for 5 min and then added 1.2mL of 5% Na_2CO_3 solution. After 2 hrs, the absorbance was recorded at 750nm. The total phenolic content was expressed as gallic acid equivalent (GAE) and calculated using equation 6. Where *C* is the content of gallic acid derived from the standard curve (mg/mL), *V* is the extract volume (mL), *m* is the sample weight (g), *k* is the dilution factor.

$$\text{Phenolic (mgGAE/100g)} = \frac{C \times V}{m} \times k \times 100 \quad (6)$$

Data analysis

Experimental data were analyzed using the Analysis of variance (ANOVA) technique to show the level of statistical significance of two independent variables (blanching temperature and time) on responses by the P-value (probability) with the 95% confidence level and expressed through standardized Pareto charts. The quadratic equation for predicting the optimal conditions was developed (equation 7). Where Y is the predicted response; β_o is the constant; β_i, β_{ii} and β_{ij} are coefficients of the variable for linear, quadratic and interaction terms, respectively; X_i, X_j are two independent variables. The quality of polynomial models was expressed by the coefficient of determination (R^2) and the P-value of lack-of-fit. The interaction between two variables and responses were obtained in three-dimensional plots and their respective contour plots. Finally, the overlay plot of all responses was used to identify the overall optimum region.

$$Y = \beta_o + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 + \sum_{i=1}^{1} \sum_{j=2}^{2} \beta_{ij} X_i X_j \quad (7)$$

Results and discussion

The standardized Pareto charts from Fig. 1 compared the effects of each variable, quadratic values and interaction term on each response. The display order of the bars from top to bottom of charts corresponded

to the order of the effect levels from the strongest to the weakest. A vertical line on charts indicated the statistical significance limit which corresponded to the 95% confidence (P=0.05). An effect was considered to be significant when the variable horizontal bar crossed this vertical line. It could be seen that both blanching variables (temperature and time) were significant for all responses because the P-values were lower than 0.05.

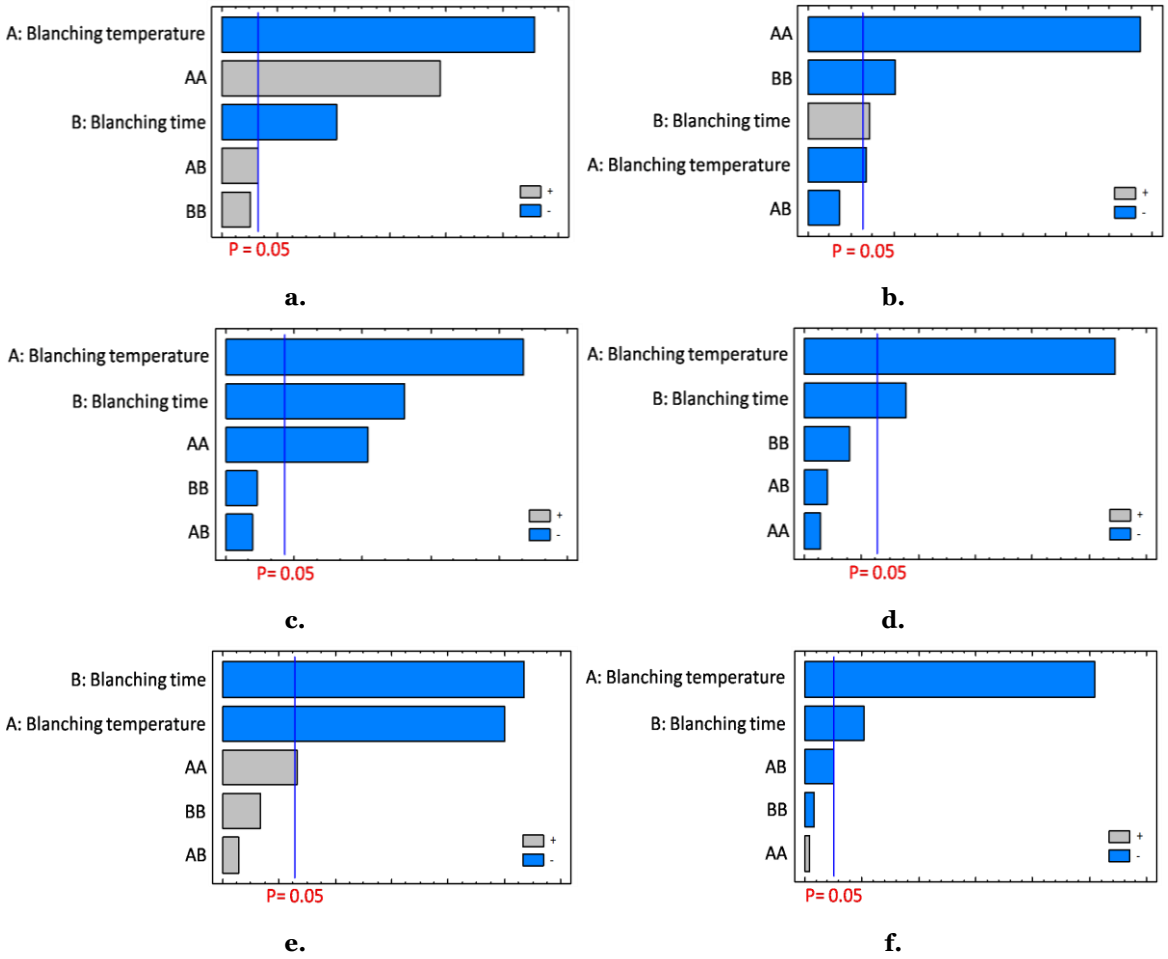

Fig. 1. Standardized Pareto charts of effects for responses.
a. Remaining POD activity; b. Pectin content, c. Anthocyanin content; d. Lycopene content; e. Vitamin C content; f. Total phenolic content

The mathematical formulations expressing the relationship between the predicted responses and two independent variables were established (from equations 8 to 13 in Table 2). The coefficient of determination (R-*squared* - R^2) indicated the percentage of variability that the chosen model explained in every response. The adjusted R-squared value was more suitable for comparing models with different numbers of independent variables. The models were considered to be fitted with experimental data when the R^2 reached at least 0.8 (Guan and Yao, 2008) and this value close to 1 was desirable.

Also, the test for lack-of-fit was carried out by comparing the variability of the model residuals to the variability between observations to check whether the chosen model was adequate to describe the experimental data. Since the P-values for lack-of-fit were greater than 0.05, the developed models appeared to be adequate for the observed data at the 95.0% confidence level and could be applied to predict the change of process responses based on two variables with high accuracy. The response surfaces and contour plots of blanching conditions were shown in Fig. 2. The two-dimensional representation of the responses on

the blanching temperature - blanching time plane (contour plot) showed concentrically closed curves whose centers represent the optimum conditions.

Many enzymes present in plant cells such as peroxidase, polyphenol oxidase, lipoxygenase or pectinase can catalyze different biochemical reactions during processing leads to a negative effect on the organoleptic and nutritional properties of products (Ciou *et al.*, 2011). In which, POD is well-known as one of the most heat-stable enzymes in fruits and vegetables, therefore it is often selected as the indicator enzyme for blanching to monitor the heat treatment process (Zhu *et al.*, 2010) because if POD is inactivated, other enzymes that cause quality problems will also be destroyed (Akyol *et al.*, 2006). The response surface plot from Fig. 2a showed a reduction in remaining POD activity along with an increase in blanching temperature and time.

Meanwhile, the soluble pectin content which was another parameter to evaluate the effectiveness of the blanching process changed in the opposite trend at temperatures lower than 90^{o}C and time is shorter than the 90s (Fig. 2b). That meant the blanching process improved soluble pectin content attributed to the conversion from protopectin under the effect of high temperature in blanching (Ramos-Aguilar *et al.*, 2015). Exceeding this limit, by contrast, the pectin content decreased due to excessive blanching resulting in cracking of the skin of fruits leading to the loss of soluble pectin into the blanched water.

Because of the ability to inactivate enzymes and destroy some microorganisms on the surface, blanching is considered to be the most common and simple method to stabilize food, however, heat can degrade the nutritional components, especially bioactive compounds (Cruz *et al.*, 2008). As can be seen from the graphs an increase in the value of variables of the blanching process led to a reduction in the content of bioactive compounds after blanching, including anthocyanin, lycopene, vitamin C and total phenolic. In black cherry tomatoes, anthocyanins are synthesized in response to light, so that, they concentrate primarily on the outer skin, which makes the fruit look attractive but not beneficial during processing (Klee, 2013). Moreover, it is known that these pigments are easily degraded by heat (Patras *et al.*, 2010). Therefore, these components suffered a lot of loss in the blanching process (Fig. 2c) which affected the colour and biological value of fruits.

For lycopene, the heat treatment can cause changes in different directions in fruits: on the skin, thermal degradation is predominant due to rapid and easy heat transfer; on the contrary, in the flesh, the lower temperatures can be obtained that promotes the breakdown of protein-carotenoid complexes but not destruct it significantly (D'Evoli *et al.*, 2013). These authors showed that the lycopene content in cherry tomato skin decrease by 36% after heat treatment. Besides, the isomerization phenomenon of lycopene during heating was also observed as lycopene presented naturally in the *trans* form in food products converted into the *cis* forms (Lee and Chen, 2002). Both the lycopene degradation and isomerization can occur simultaneously and which process dominates depend on temperature (Lee and Chen, 2002). The above reasons explained why there was only a small reduction in lycopene content during blanching (Fig. 2d).

Vitamin C is the least stable of all vitamins and it is easily destroyed during heat processing (Cruz *et al.*, 2008) so that it lost significantly (Fig. 2e). Meanwhile, when tomatoes were subjected to blanching, the total phenolic seemed to decrease slightly (Fig. 2f). The heat treatment can contribute to the destruction of phenolics, especially at high-temperature conditions (Teh *et al.*, 2016) and the degree of degradation depends greatly on the processing time and fruit size (Aminah and Permatasari, 2013). Moreover, the dissolution of soluble phenolics into the water when blanched at high temperature and for a long time due to the phenomenon of fruit cracking was also a contributing factor to the loss of phenolics.

Table 2. The regression equations in terms of coded variables to predict the responses.

Responses	Equations		R^2	R^2 (adjusted for d.f.)	P-value (lack-of-fit)
Remaining POD activity (%)	$Y_2 = 4820.34 - 102.058X_1 - 2.6729X_2 + 0.54376X_1^2 + 0.02294X_1X_2 + 0.00254X_2^2$	(8)	0.9899	0.9837	0.4336
Pectin content (%)	$Y_3 = -172.915 + 3.82138X_1 + 0.08284X_2 - 0.02104X_1^2 - 0.0005X_1X_2 - 0.0002X_2^2$	(9)	0.9685	0.9488	0.3928
Anthocyanin content (mgCE/100g)	$Y_4 = -43.5641 + 1.06923X_1 + 0.02962X_2 - 0.00599X_1^2 - 0.00028X_1X_2 - 0.00005X_2^2$	(10)	0.9770	0.9627	0.7126
Lycopene content (µg/g)	$Y_5 = -101.178 + 3.36633X_1 + 0.94029X_2 - 0.02082X_1^2 - 0.00722X_1X_2 - 0.00198X_2^2$	(11)	0.9580	0.9317	0.8262
Vitamin C content (mg/100g)	$Y_6 = 697.257 - 12.9643X_1 - 0.69027X_2 + 0.06638X_1^2 + 0.00372X_1X_2 + 0.00119X_2^2$	(12)	0.9678	0.9477	0.5348
Total phenolic content (mgGAE/100g)	$Y_7 = 142.146 - 1.97501X_1 + 1.38181X_2 + 0.00942X_1^2 - 0.01467X_1X_2 - 0.00069X_2^2$	(13)	0.9867	0.9784	0.3481

Notes: X_1 was blanching temperature (°C), X_2 was blanching time (s)

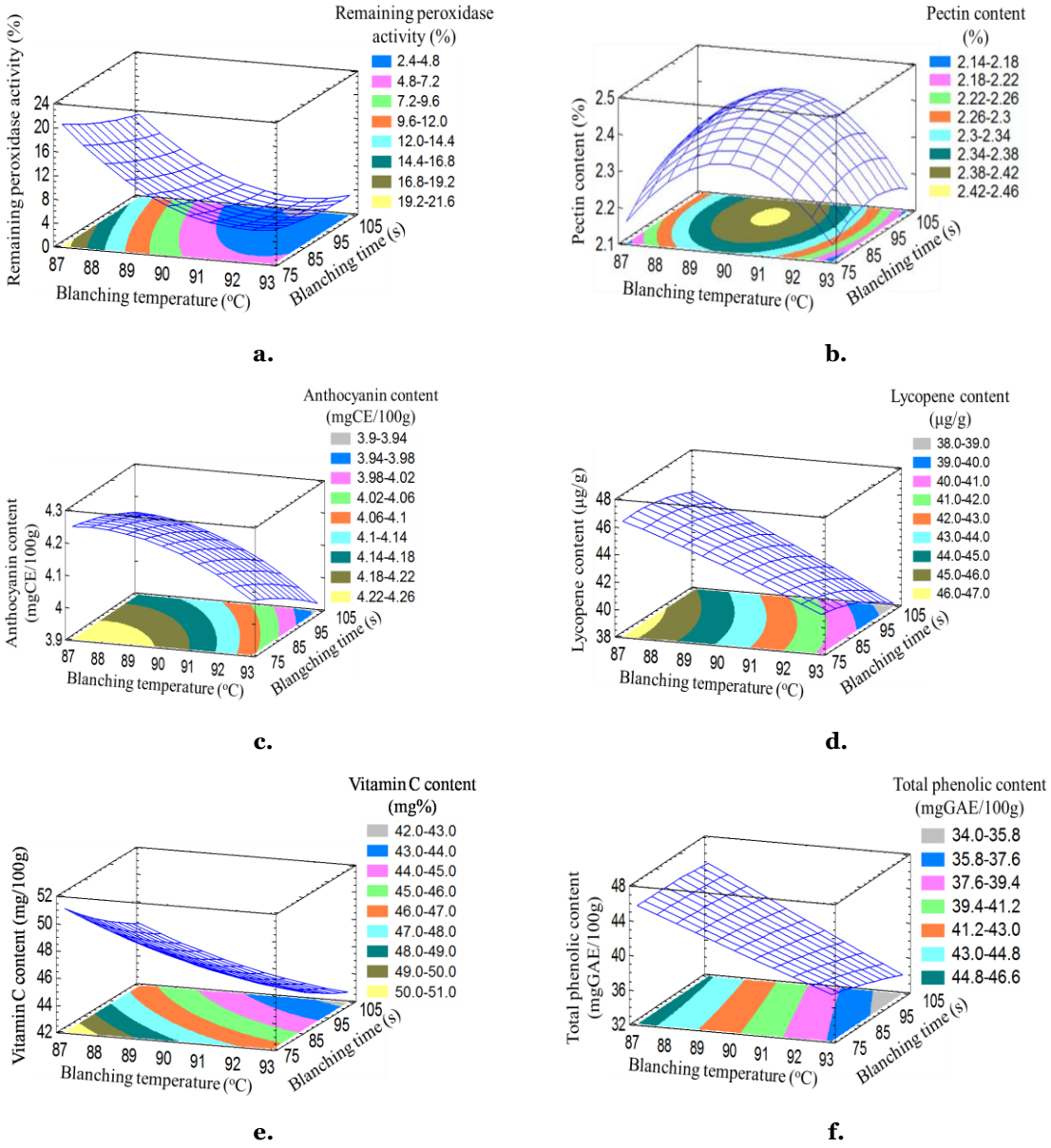

Fig. 2. Response surface and contour plots for the effects of blanching temperature and time on responses.
a. Remaining POD activity; b. Pectin content, c. Anthocyanin content; d. Lycopene content; e. Vitamin C content; f. Total phenolic content

From the models, optimum blanching conditions of black cherry tomatoes for each response were obtained in Table 3. However, each indicator reached the optimal value under different blanching conditions, therefore the overall optimum point where all responses simultaneously meet the desirable criteria could be visualized graphically by overlaying of contour plots (Fig. 3).

Table 3. Optimum conditions of blanching process for each response.

Response	Optimum value	Optimum conditions	
		Blanching temperature (°C)	Blanching time (s)
Remaining peroxidase activity (%)	2.09187	91.5035	111.0
Pectin content (%)	2.42505	89.6961	95.6002
Anthocyanin content (mgCE/100g)	4.26089	87.6487	68.0
Lycopene content (µg/g)	47.2099	86.0	80.686
Vitamin C content (mg/100g)	53.6311	86.0	68.0
Total phenolic content (mgGAE/100g)	47.2086	86.0	86.8497

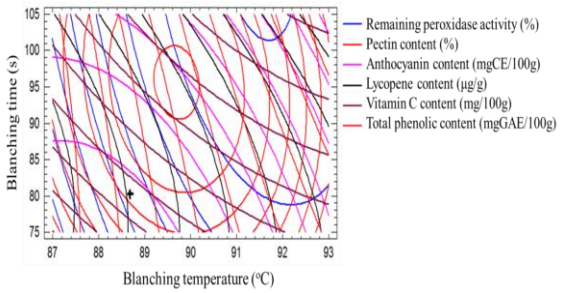

Fig. 3. Overlay plot for optimum region.

Under the optimum conditions of blanching temperature 88.68°C and blanching time 80.28s, a minimum response of 11.11% remaining POD activity and maximum responses of 2.35% pectin, 4.23mgCE/100g anthocyanin, 44.93 µg/g lycopene, 48.39mg/100g vitamin C and 43.14mgGAE/100g total phenolic were predicted. An experiment was carried out applying the above optimum conditions and the data obtained for indicators of remaining POD activity along with pectin, anthocyanin, lycopene, vitamin C and total phenolic content were 10.06%, 2.36%, 4.22mgCE/100g, 44.64 µg/g, 46.61mg/100g and 42.68mgGAE/100g, respectively. This confirmed the agreement of the results achieved from models and experiments.

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