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Improvement the firmness of thermal treated black cherry tomatoes (*Solanum lycopersicum* cv. OG) by low-temperature blanching in calcium chloride solution

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Abstract

The objective of this study was to investigate the effects of low-temperature blanching in calcium chloride ($CaCl_2$) solution before high-temperature processing (90°C for 1 min) on physicochemical properties related to the texture (total calcium content, pectin methylesterase (PME) activity, and firmness) of black cherry tomatoes (*Solanum lycopersicum* cv. OG) and to determine the optimum conditions to provide the fruits with the highest firmness. Response Surface Methodology (RSM) was applied to design the experiment with three factors of blanching temperature (52 to 68°C), blanching time (12 to 28 mins) and $CaCl_2$ solution concentration (1.2 to 2.8% w/v). A sample without low-temperature treatment was taken as a control. It was found that the blanching of tomatoes at low-temperatures resulted in higher total calcium content and PME activity in tissue after the treatment, and therefore improved the firmness of fruits after high-temperature processing. The highest firmness of treated tomatoes (566.21 g force) was estimated at blanching temperature of 62.05°C for 23.09 mins in 2.08% $CaCl_2$ solution compared to the control which was blanched at high temperature (218 g force). Under these optimized conditions, the experimental responses (total calcium content, PME activity, and firmness) showed a close agreement with the predicted values.

1. Introduction

"Black" or "purple" cherry tomatoes are subspecies of *Solanum lycopersicum* (Zhang *et al*., 2018) and exhibit a purplish-brown color on their skin (Mes *et al*., 2008). Black cherry tomatoes contain a variety of bioactive compounds such as lycopene, vitamin C, and also anthocyanin (Li *et al*., 2011). Because of their health benefits, black cherry tomatoes can be used as a fresh vegetable or processed into many products (Zhang *et al*., 2018).

An important quality attribute of processed tomatoes is texture. Upon processing, changes in tomato texture are due to changes in structure and chemical composition. The rigid structure of the raw tomato is mainly due to the pectin substances, celluloses, and hemicelluloses. The pectic substances, which are the main constituents of the middle lamella, play a major role in intercellular adhesion and also contribute to the mechanical strength of the cell wall (Abu-Ghannam and Crowley, 2006). Conventional blanching of fruits and vegetables, which is carried out within the range of 80-100°C for short times to inactivate undesirable enzymes (Abu-Ghannam and Crowley, 2006), is the main pretreatment that precedes further techniques such as drying, canning, and freezing (Ahrné *et al*., 2003). However, the thermal operations applied in the production of fruits and vegetables frequently result in a significant loss of textural integrity. The most immediate effect of high temperatures is a loss of turgor pressure (Ni *et al*., 2005). One of the approaches to avoid loss of texture is to use calcium salts, which bind with pectic substances to form calcium pectate, making fruits and vegetables more resistant to acid hydrolysis and thermal softening (Pérez-Alemán *et al*., 2005). Calcium salts also bind to free carboxylic acid groups along the polygalacturonic acid backbone of the pectin to form cross-links between pectin chains in the middle lamella leads to greater adhesion between cells and a firmer texture (Anthon *et al*., 2005).

On the other hand, many previous studies which aimed at improving the texture of processed fruits and vegetables also found that preheating them at low-temperature for a long time (in a so-called low-

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temperature blanching) results in a firmer product through subsequent high-temperature processing (Stolle-Smits *et al*., 2000; Ni *et al*., 2005; Abu-Ghannam and Crowley, 2006). This is due to the activation of enzyme pectin methylesterase (PME) which is present in the cell wall when the fruits and vegetables are soaked in water at a temperature of 50-70^{o}C for periods of 15-90 mins (Quintero-Ramos *et al*., 2002). The beneficial effect on the firmness of processed fruits and vegetables has been observed to intensify if calcium treatment was used in combination with low-temperature blanching (Domínguez *et al*., 2001; Zhao *et al*., 2016). PME hydrolyzes the methyl ester linkages in pectin molecules, releasing methanol and free galacturonic acid moieties (Ni *et al*., 2005). The resulting free carboxyl groups between pectin polymers may then form cross-links through the salt-bridge formation with divalent cations (notably Ca^{2+}) naturally present in the tissue or added to the blanching solution (Christiaens *et al*., 2012). PME also catalyzes a transacylation reaction of the galacturonic acyl groups from methanol to other hydroxyl groups of pectin, resulting in the formation of new ester linkages between pectin molecules and thus contributing to tissue firming (Ni *et al*., 2005). Besides, Anthon *et al*. (2005) observed that heating diced tomatoes to 70^{o}C either before or after the $CaCl_2$ treatment also improved firmness through a subsequent high-temperature treatment, but to a lesser extent than heating during the $CaCl_2$ treatment.

This study was carried out for a new cultivar of black cherry tomato (*Solanum lycopersicum* cv. OG) grown in Vietnam to evaluate how blanching temperature, time after exposure, and concentration of calcium chloride ($CaCl_2$) solution (blanching medium) affected the uptake of calcium, the activity of pectin methylesterase (PME), and the firmness of the whole fruit. Also, the present work identified the conditions of low-temperature blanching at which the firmness of fruits after conventional thermal processing is at optimum value.

2. Materials and methods

2.1 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) corresponding to the diameter of fruits was 25.11±1.83 mm, the total solid content and pH value were 6.17±0.12^{o}Brix and 4.43±0.06, respectively. All fruits with diseases and defects were removed. Fruits were packed into perforated polyvinyl chloride and then cardboard boxes. They were transported to the Food Technology Laboratory of Can Tho University, Vietnam within 1 hr. Tomatoes were washed and immersed in ozonated water for 15 mins to kill microorganisms on the surface (using a 2-nozzle ozone generator, model Z755, Vietnam, ozone-generating of 80.4 mg/h , the sample weight of 1500 g, the ratio of fruits and water was 1:2).

The infiltration process was then carried out in the vacuum equipment (Rocker 400, Laftech, Australia) to increase the thermal conductivity during subsequent blanching due to the replacement of gases inside the fruit spores by the liquid. The vacuum level and treatment time were chosen as 620 mmHg and 22 mins, respectively, and a ratio of material and distilled water was 1:1. After vacuum infiltration, the mixture of fruits and water was brought to the atmospheric condition and kept for a further 15 mins. Fruits were drained and used for the blanching experiment with the firmness of 899 g force, the calcium content of 19.17 mg/100 g fresh material, and the PME activity of 1.22 U/mL.

2.2 Experimental design

The low-temperature blanching experiment was designed by the Portable Statgraphics Centurion software (version 15.2.11.0, U.S.A.). The Response Surface Methodology (RSM) with a model of Central Composite Design (CCD) was applied. Blanching temperature (X_1), time after exposure (X_2), and $CaCl_2$ (Merck, Germany) concentration in the blanching medium (X_3) were three independent variables. Before designing the optimization experiment, a preliminary investigation was carried out for a wide range of blanching temperature (50-70^{o}C), blanching time (10-30 mins), $CaCl_2$ concentration (1-3%) and as a result, the narrower study ranges were chosen as 52-68^{o}C, 12-28 mins, and 1.2-2.8%. The actual and coded values of each variable were presented in Table 1. Each variable was encoded with five levels: -1.68179, -1 (low), 0 (central), +1 (high) and +1.68179. The total number of runs was 20, including six replications of the central point. Tomatoes (1 kg) were put into a stainless steel rectangular mesh basket (a length of 25.5 cm, a width of 10 cm, and a height of 6.5 cm) with a square hole size of 0.5 cm) and soaked into a thermostatically controlled water bath (Rex C-90, Memmert, Germany) containing $CaCl_2$ solution, in which the ratio of material and $CaCl_2$ solution was 1:2. The preheated samples were then subjected to a second blanching in the water at 90^{o}C for 1 min (high-temperature blanching) to inactivate enzymes which can generate undesirable changes in color and odor during subsequent processing and cooled quickly by immersion in cold water (10^{o}C) for 1 min. The control sample was

given a single blanching in the water at 90°C for 1 min and then cooled. The total calcium content and PME activity of tomatoes after preheating and the firmness of fruits after high-temperature processing were evaluated.

2.3 Experimental verification

After analyzing the data, black cherry tomatoes were treated following the above procedure with selected optimum parameters of low-temperature blanching in triplicate to verify the results under experimental conditions. The responses were measured and compared with the predicted values from the proposed mathematical model.

2.4 Analytical methods

2.4.1 Firmness

Fruit firmness was determined with a RheoTex (SD 700, Sun Science, Japan). A 1 cm - diameter cylindrical probe with a flat end was used in this case. The maximum compression force required to press vertically into the middle of fruits for a 4 mm travel distance was measured and expressed in g force, using a constant speed of 1 mm/s. Five replicates of each sample were carried out and the mean was calculated.

2.4.2 Pectin methylesterase (PME) activity

PME activity was determined directly using the method described by Ni *et al*. (2005). The enzyme was extracted from tomatoes by homogenizing with 2 volumes (w/v) of 0.2 M NaCl for 1 min. The mixture was shaken for 30 mins and then filtered through filter paper. A 0.5 mL of filtrate was mixed with 0.25% pectin -salt (0.2 M NaCl) substrate. The mixture was immediately adjusted to pH 7.0 with 0.1 M NaOH. After the initial adjustment, the mixture was shaken and 0.1 M NaOH was added quantitatively until pH 7.0 was re-established. The time was measured until the pH of the mixture regained pH 7.0. A blank titration was carried out under the same conditions, but 0.5 mL of 0.2 M NaCl solution was used instead of the tomatoes filtrate. PME activity was calculated by the equation 1, where n is the molarity of NaOH solution, V_{NaOH} is the volume of 0.1 M NaOH solution used for titration (mL), V_{sample} is the volume of sample (mL), and t is the titration time (min).

$$PME\ (U/mL) = n \times \frac{V_{NaOH}}{V_{sample}} \times t \quad (1)$$

2.4.3 Total calcium content

The total calcium content was determined by the method of precipitate formation with ammonium oxalate (So and Thuan, 1975). The 5 g of tomato puree was dried in a furnace at 550°C until white ash was obtained. The ash was then dissolved in 5 mL of 20% HCl and the solution was filtered through a filter paper. The filtrate was filled to a volume of 100 mL with distilled water. The diluted filtrate (25 mL) was added 2 mL of saturated NH_4Cl and drop-by-drop of 20% NH_4OH until obtaining an alkaline reaction. After filtering out the precipitate, the solution was then added 2 drops of red methyl and acidified with 20% CH_3COOH until a light pink color appeared. After boiling, the mixture was added 10 mL of saturated ammonium oxalate. The mixture was boiled again and cooled to form the precipitate of calcium oxalate. After 1 hr, the mixture was filtered through a filter paper. The filter paper containing the precipitate was taken into a conical flask with 25 mL of 20% H_2SO_4. The mixture was then heated to 80°C and titrated with 0.02 N $KMnO_4$ until a pink color appeared. Total calcium content was calculated using equation 2, where K is the weight of Ca^{2+} corresponding to 1 mL of $KMnO_4$ (K = 0.1 mg); n is the volume of $KMnO_4$ used for titration; m is the weight of the sample (g).

$$Ca\ (mg/100\ g) = K \times n \times \frac{100}{25} \times \frac{100}{m} \quad (2)$$

2.5 Data analysis

Experimental data were analyzed by the Portable Statgraphics Centurion software (version 15.2.11.0, U.S.A.). The statistically significant effect of three independent variables (blanching temperature, blanching time, and $CaCl_2$ concentration) on responses was expressed through standardized Pareto charts at the 95% confidence level (the P-value (probability) less than 0.05). The second-order polynomial equation for predicting the optimum conditions was developed (equation 3), 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 and X_j were 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.

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

3. Results and discussion

3.1 Effect of the low-temperature blanching on responses

The influence of temperature, time, and $CaCl_2$ concentration during low-temperature blanching on the total calcium content, PME activity, and firmness of whole tomato fruit was illustrated in Table 1. The standardized Pareto charts from Figure 1 compared the linear and quadratic effects and interaction on each response. The display order of the bars from top to bottom of charts corresponded to the order of the effect

Table 1. Total calcium content, PME activity, and firmness of whole tomato fruit at different blanching conditions

Run	Blanching temperature (°C)	Blanching time (min)	$CaCl_2$ concentration (%)	Total calcium content (mg/100 g)	PME activity (U/mL)	Firmness (g force)
1	60 (0)	20 (0)	2.0 (0)	67.71	3.75	516
2	60 (0)	20 (0)	2.0 (0)	67.41	3.72	557
3	60 (0)	20 (0)	2.0 (0)	67.22	3.71	550
4	60 (0)	12 (-1.68179)	2.0 (0)	65.78	2.18	435
5	60 (0)	20 (0)	2.8 (+1.68179)	66.16	2.25	447
6	65 (+1)	15 (-1)	1.5 (-1)	69.36	3.43	459
7	68 (+1.68179)	20 (0)	2.0 (0)	77.36	3.84	464
8	52 (-1.68179)	20 (0)	2.0 (0)	59.18	1.71	232
9	60 (0)	20 (0)	2.0 (0)	66.96	3.67	545
10	65 (+1)	25 (+1)	1.5 (-1)	72.19	3.71	484
11	60 (0)	28 (+1.68179)	2.0 (0)	66.57	2.56	551
12	55 (-1)	25 (+1)	1.5 (-1)	62.29	1.79	374
13	65 (+1)	15 (-1)	2.5 (+1)	73.26	2.75	515
14	55 (-1)	15 (-1)	2.5 (+1)	62.66	1.88	291
15	65 (+1)	25 (+1)	2.5 (+1)	74.24	2.86	499
16	60 (0)	20 (0)	2.0 (0)	66.84	3.63	542
17	55 (-1)	15 (-1)	1.5 (-1)	59.45	1.94	275
18	60 (0)	20 (0)	2.0 (0)	66.32	3.58	536
19	55 (-1)	25 (+1)	2.5 (+1)	63.01	1.91	404
20	60 (0)	20 (0)	1.2 (-1.68179)	63.54	2.94	407
		Control		19.17	1.22	218

Numbers in parentheses were coded values.

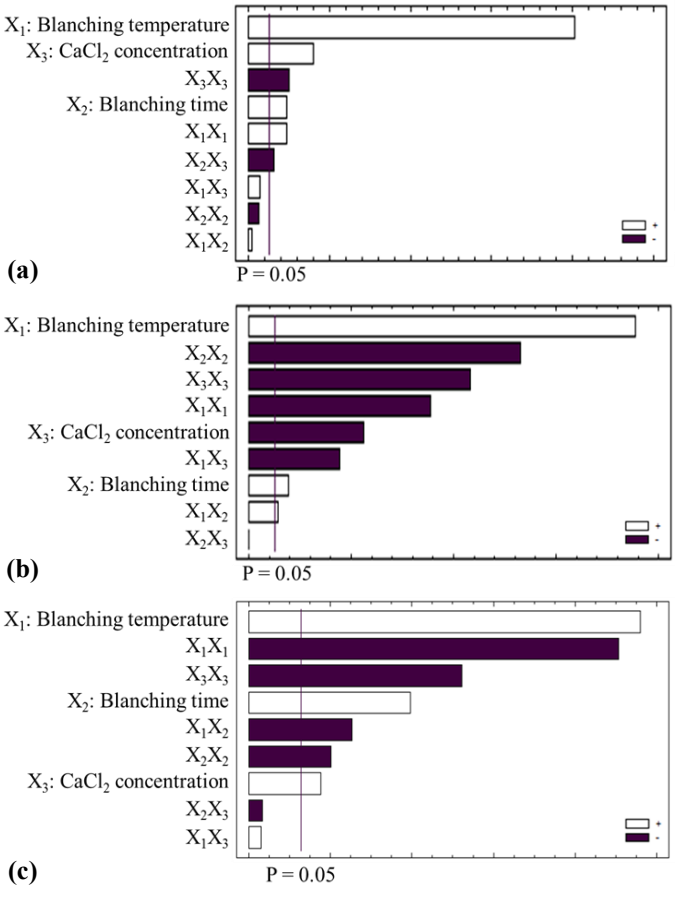


Figure 1. Standardized Pareto charts of effects for responses of (a) total calcium content (mg/100 g), (b) PME activity (U/mL), (c) firmness (g force)

levels from the strongest to the weakest. A blue 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 all studied variables (blanching temperature, blanching time, and $CaCl_2$ concentration) had a marked linear effect on total calcium content, PME, and firmness because the P-values were lower than 0.05, in which, blanching temperature was the most important factor.

3.2. Predicted models for responses

The mathematic formulations expressing the relationship between the predicted responses and three independent variables were established (from Equation 4 to 6 in Table 2). The coefficient of determination (R-*squared* - R^2), which indicated the percentage of variability that the chosen model explained in every response, was used to assess the model satisfactoriness. 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. In these responses, adequate agreements were obtained between empirical and predicted data from second-order models, which were indicated by high correlation coefficient values (R^2>0.98). This would be

Table 2. 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)
Total calcium content (mg/100 g)	$Y_1 = 81.8639 - 2.28551X_1 + 0.65013X_2 + 11.4679X_3 + 0.02583X_1^2 + 0.0031X_1X_2 + 0.101X_1X_3 - 0.0069X_2^2 - 0.217X_2X_3 - 2.76069\ X_3^2$	(4)	0.9876	0.9765	0.1005
PME activity (U/mL)	$Y_2 = -69.7202 + 1.76383X_1 + 0.62056X_2 + 10.5731X_3 - 0.0127X_1^2 + 0.00255X_1X_2 - 0.0795X_1X_3 - 0.01903X_2^2 + 0.0005X_2X_3 - 1.55138X_3^2$	(5)	0.9903	0.9815	0.0528
Firmness (g force)	$Y_3 = -13088 + 382.627X_1 + 95.7812X_2 + 651.922X_3 - 2.9152X_1^2 - 1.015X_1X_2 + 1.25X_1X_3 - 0.64958X_2^2 - 1.35X_2X_3 - 168.083X_3^2$	(6)	0.9891	0.9794	0.5133

Notes: X_1 was blanching temperature (°C), X_2 was blanching time (min), X_3 was $CaCl_2$ concentration (%)

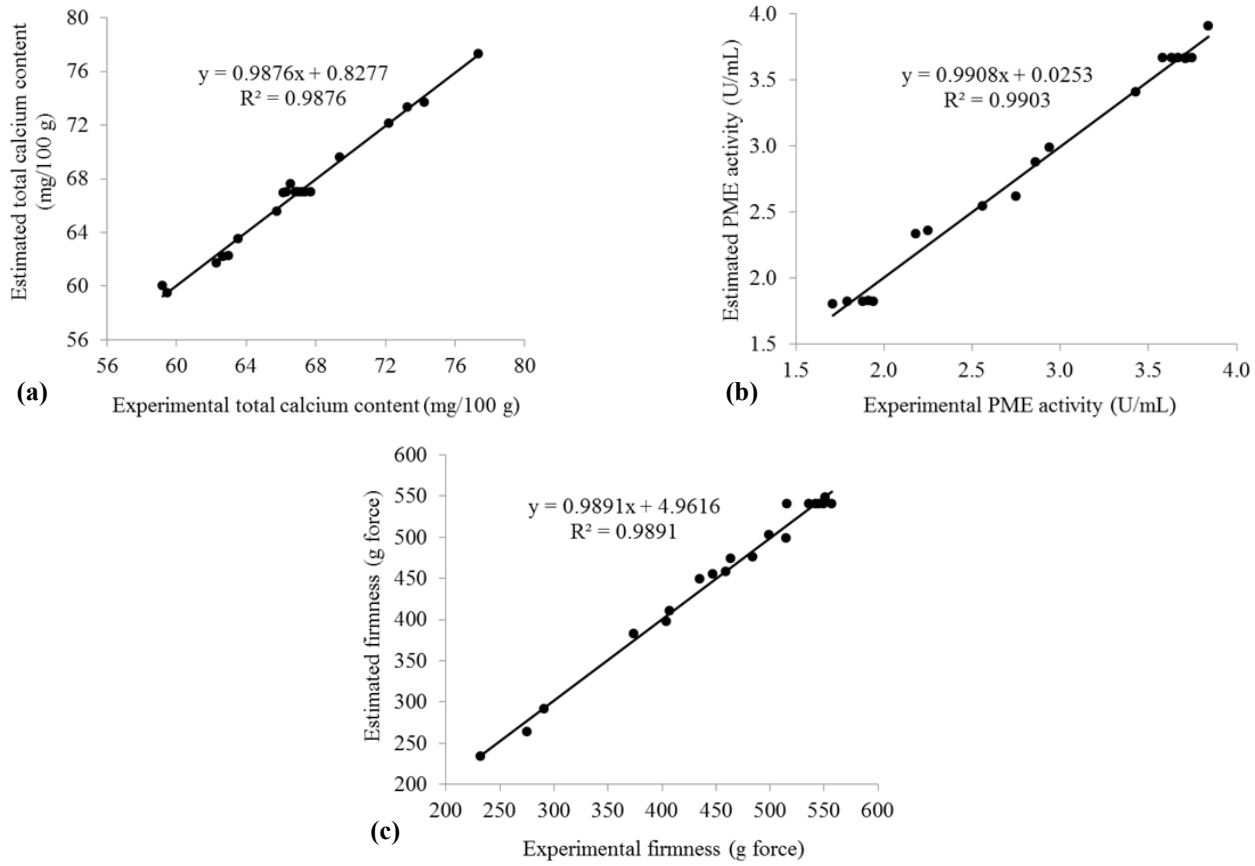


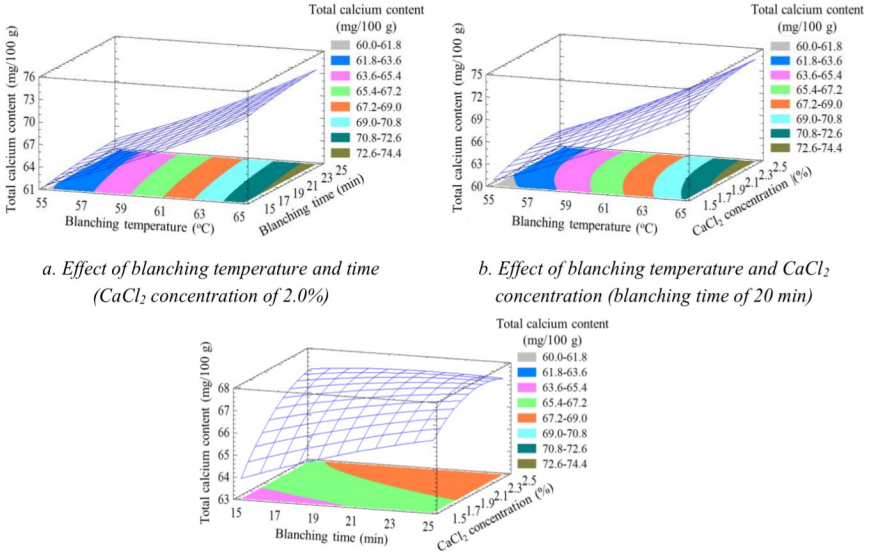
Figure 2. Correlation between the experimental and estimated values for responses of (a) total calcium content, (b) PME activity and (c) firmness.

illustrated clearly by the scatter plots of the estimated versus experimental values (Figure 2). Besides, 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 fitted to the observed data at the 95% confidence level and could be applied to predict the change of process responses based on three variables with high accuracy.

3.3 Response surfaces and contour plots for responses

The response surfaces and contour plots of blanching conditions were shown in Figures 3, 4, and 5. The two-dimensional representation of the responses on every two -variables plane (contour plot) showed concentrically closed curves whose centers represented the optimum conditions.

As can be seen from Figure 3, the calcium absorption increased as the blanching temperature, blanching time, and $CaCl_2$ concentration in the blanching medium increased. Such behavior was in agreement with the observations during low-temperature blanching of carrot slices (Quintero-Ramos *et al*., 2002) and Jalapeno pepper (Pérez-Alemán *et al*., 2005) in calcium chloride solution. This has been due to the alteration of cell wall permeability that is related to the mass transfer of calcium in the tissue, and also to a possible reaction of calcium ions with free carboxylic groups coming from the de-esterification of pectins in the process (Pérez-

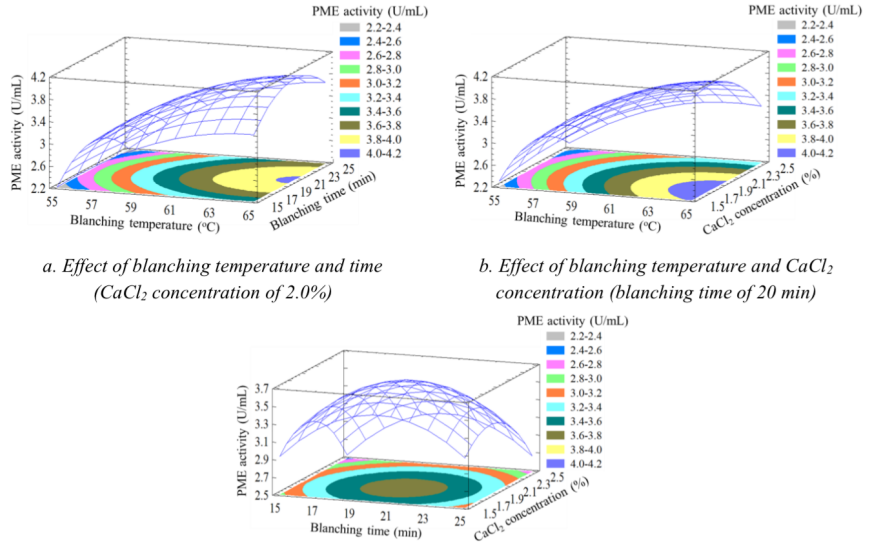


a. Effect of blanching temperature and time (CaCl₂ concentration of 2.0%)

b. Effect of blanching temperature and CaCl₂ concentration (blanching time of 20 min)

c. Effect of blanching time and CaCl₂ concentration (blanching temperature of 60°C)

Figure 3. Response surface and contour plots for the effect of low-temperature blanching variables on the total calcium content of tomatoes after treatment

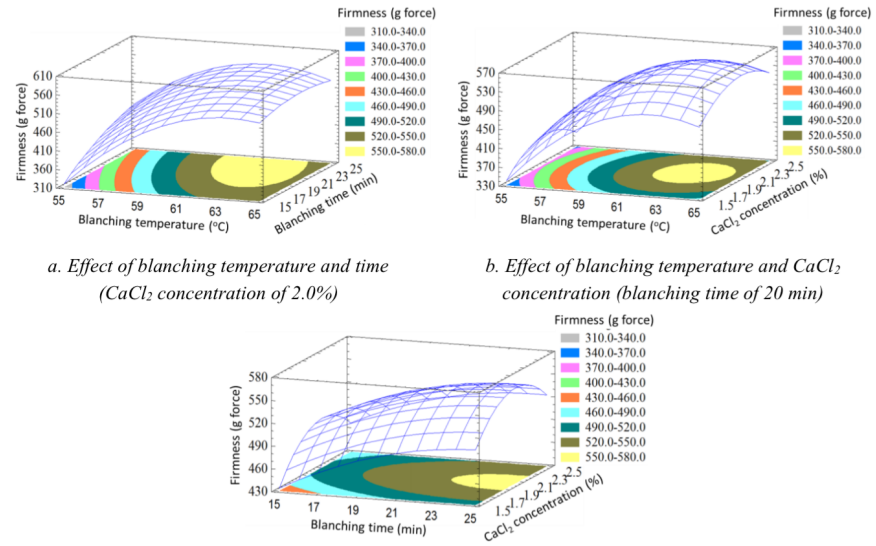


a. Effect of blanching temperature and time (CaCl₂ concentration of 2.0%)

b. Effect of blanching temperature and CaCl₂ concentration (blanching time of 20 min)

c. Effect of blanching time and CaCl₂ concentration (blanching temperature of 60°C)

Figure 4. Response surface and contour plots for the effect of low-temperature blanching variables on the PME activity of tomatoes after treatment



a. Effect of blanching temperature and time (CaCl₂ concentration of 2.0%)

b. Effect of blanching temperature and CaCl₂ concentration (blanching time of 20 min)

c. Effect of blanching time and CaCl₂ concentration (blanching temperature of 60°C)

Figure 5. Response surface and contour plots for the effect of low-temperature blanching variables on the firmness of tomatoes after high-temperature processing

Alemán *et al*., 2005). The optimum conditions (blanching temperature of 68°C, a blanching time of 26.58 mins, and a $CaCl_2$ concentration of 2.28%) resulted in the highest total calcium content in the tissue (78.20 mg/100 g) (Table 3), which was significantly higher ($P<0.05$) than those treated at 90°C for 1 min without blanching (19.17 mg/100 g). After the increase in a blanching time of 15 to 26.58 mins and a concentration range of 1.5 to 2.28% $CaCl_2$, very little additional calcium content was observed.

It was evident that low-temperature blanching improved the PME activity of tomatoes after blanching (Figure 4). Optimum activities reported for PME in different studies varies due to variations in composition and variety, however, all reported values lie in the range of 50-70°C (Abu-Ghannam and Crowley, 2006). The effects of calcium and other cations on PME activity are through the interactions of the cations with the pectin substrate rather than a direct effect on the PME enzyme. As PME de-esterifies the pectin, the number of free carboxyl groups in the pectin increases, causing the enzyme to become tightly bound to the pectin and thus inactive. Added cations such as calcium compete for these binding sites, reducing the binding of the enzyme to the pectin, and allowing for additional de-esterification to occur (Anthon *et al*., 2005). Calcium also inhibits polygalacturonase activity, therefore, if heating is done in the absence of calcium, some of the de -esterified pectins will be cleaved by polygalacturonase activity, reducing cell wall strength and thus decrease firmness (Anthon *et al*., 2005).

Optimum activity for PME in this study (4.13 U/mL) was observed at 66.15°C after a 20.76 mins blanching period in 1.72% $CaCl_2$ solution (Table 3). However, further increases in these variables produce a decrease in the activity of this enzyme. Partial inactivation of PME would explain why the measured activity was lower at higher temperatures and longer times. As we have shown previously, in tomato juice, thermal inactivation of PME becomes significant at temperatures above 70°C (Anthon *et al*., 2002). The activity of PME, purified from tomatoes and acting on pure pectin, was known to be increased by added calcium, but with a narrow optimum above which additional calcium inhibits (Lee and Macmillan, 1968). Increasing the level of PME activity could increase the number of calcium-binding sites in the pectins and thus allow for increased calcium cross-linking and a better texture (Anthon *et al*., 2005).

Upon investigating the effect that blanching temperatures used had on the texture of potatoes, Abu-Ghannam and Crowley (2006) found that samples blanched at 65°C and treated at 100°C for 25 mins gave a higher firmness than those blanched at 75°C and then treated at 100°C for 25 mins, however, when the texture was measured after blanching, i.e. before processing, there was no significant difference between shear force values for 65 and 75°C. It would appear that the firming effect of low temperature blanching only becomes evident after the subsequent thermal processing. A similar finding was achieved by Ni *et al*. (2005) whereby the firmness of cabbage, green bell peppers, sugar snap peas, carrot, and broccoli were enhanced when processing was preceded by a blanching step. Therefore, in this study, the firmness of fruits was also determined after the conventional blanching (as would be carried out in the industry).

The firmness of fruits also increased as the experimental conditions were elevated (Figure 5). The maximum value of 566.21 g was reached at a blanching temperature of 62.05°C for 23.09 mins in 2.08% $CaCl_2$ solution (Table 3). The experimental conditions above these values produced a decrease in the firmness. The maximum firmness was 2.61 times higher than the control sample (218 g force). The firming effect resulting from a low-temperature treatment may be attributed to the stimulation of enzyme pectin methylesterase (PME), which is activated at temperatures between 50 and 70°C (Ni *et al*., 2005) coupled with the increased cross-linking to produce insoluble pectate from the calcium absorbed during the blanching (Quintero-Ramos *et al*., 2002). However, the change in the firmness was not as great as those obtained with calcium absorption, which indicated that an excess of calcium ions in the fruits did not improve the firmness significantly. These results exhibited a similar trend to those reported by Quintero-Ramos *et al*. (2002) for carrots and Pérez-Alemán *et al*. (2005) for jalapeño pepper.

Each response reached the optimum value under different blanching conditions. However, as indicated in the objective, the firmness should be as high as possible, therefore, this study chose the optimum conditions of a

Table 3. Optimum conditions of low-temperature blanching for each response

Responses	Optimum value	Optimum conditions		
		Blanching temperature (°C)	Blanching time (min)	$CaCl_2$ concentration (%)
Total calcium content (mg/100 g)	78.2	68	26.58	2.28
PME activity (U/mL)	4.13	66.76	20.76	1.72
Firmness (g force)	566.21	62.05	23.09	2.08

blanching temperature of 62.05°C for 23.09 mins in 2.08% $CaCl_2$ solution Under these conditions, the firmness of 566.21 g force after high-temperature processing with the total calcium content of 69.74 mg/100 g fresh fruits and the PME activity of 3.70 U/mL after blanching was predicted.

3.4 Empirical validation of the predicted models

The obtained results were finally verified. With optimum conditions selected, the experimental firmness, total calcium content, and PME activity were 563.26 g force, 69.56 mg/100 g, and 3.81 U/mL, respectively. These results confirmed that experimental values were in agreement with the predicted values, thus the models were validated.

4. Conclusion

The low-temperature treatment for longer time in $CaCl_2$ solution which applied for black cherry tomatoes (cv. OG) resulted in higher total calcium content and PME activity of whole fruit after soaking. Consequently, the firmness of tomatoes was improved after blanching at 90°C for 1 min as compared to conventional blanching alone. The high value of firmness of fruit was attributed to the combined action of PME and the addition of calcium. The response surface methodology was effective in the optimization of tomatoes treatment. The maximum firmness was 566.21 g force (only reduced 37%) compared to 217 g force of control sample (reduced 76%) from the initial value of 899 g. The second-order polynomial models for the responses were verified experimentally and provided a satisfactory fit.

Conflict of interest

The authors declare no conflict of interest.

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