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EFFECT OF CALCIUM CHLORIDE TREATMENT BY VACUUM INFILTRATION METHOD ON TEXTURE AND SHELF LIFE OF BLACK CHERRY TOMATOES (*Solanum lycopersicum* cv. OG)

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ABSTRACT

The use of vacuum infiltration in the food industry has several advantages, such as improving the quality, eliminating chemical treatment requirements, stabilizing products, and retaining nutrients during storage and processing. This study was performed to optimize the vacuum level (516-684 mmHg), treatment time (10-20 min), and calcium chloride ($CaCl_2$) concentration (0.58-1.42%) using the Response Surface Methodology (RSM). The firmness of black cherry tomatoes (*Solanum lycopersicum* cv. OG) reached an optimum value (1477.81 g/cm^2) when fruits were treated at a vacuum level of 637 mmHg with a concentration of $CaCl_2$ 1.09% for 17 min. Meanwhile, the firmness was 746 g/cm^2 for the control sample, which was dipped in 1.42% $CaCl_2$ solution for 20 min at the atmospheric condition. The scanning electron microscope (SEM) images of stomata of two samples with/without vacuum treatment were also significantly different. The sample after vacuum treatment at optimum parameters was put into PE and PP bags for storage at 10-12°C. Vacuum-infiltrated tomatoes contained in PE and PP bags had a corresponding storage time of 30 and 28 days. The control samples were maintained for shorter periods, only 22 and 20 days, respectively.

Key words: Black cherry tomato, vacuum infiltration, calcium chloride, firmness, SEM image, shelf-life

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a common vegetable that can either be used in fresh form or an ingredient in many processed products (Toor & Savage, 2005). 'Black' or 'purple' is a name often applied to varieties exhibiting a purplish-brown color (Mes *et al*., 2008). In addition to the known bioactive compounds, purple tomatoes also contain anthocyanin (Li *et al*., 2011). Anthocyanin has been proven to be associated with many health benefits (Lila, 2004).

Tomatoes are easily perishable during harvesting, transportation, and storage (Pila *et al*., 2010). If proper post-harvest technology (fruit handling, packaging, and storage) is not available, tomatoes not only reduce their quality but also lose their weight significantly (Pila *et al*., 2010). Approximately 20-50% of fresh tomatoes were lost at harvest and post-harvest stages in tropical countries (Pila *et al*., 2010). Calcium has gained a lot of attention due to its high effectiveness in delaying the ripening and senescence as well as reducing physiological disorders and extending the storage time (Bhattarai & Gautam, 2006). The ripening could be delayed when increasing calcium chloride ($CaCl_2$) concentration for tomato treatment (Senevirathna & Daundasekera, 2010).

Vacuum impregnation is considered a treatment to incorporate functional components into the porous structure of fruit and vegetables, in which, the driving force of the mass transfer process is due to the pressure difference (Radziejewska-Kubzdela *et al*., 2014). The storage time of many types of fruit and vegetables such as apples (Scott & Wills, 1979), avocados (Wills *et al*., 1988), strawberries (Ponappa *et al*., 1993), lemons (Valero *et al*., 1998), tomatoes (Senevirathna & Daundasekera, 2010) and grapes (Mao *et al*., 2017) has been improved by the application of vacuum technique during the

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infiltration of fruits in preservative solutions. Many studies on vacuum infiltration showed the feasibility and validity of the combination of hydrodynamic mechanism and deformation–relaxation phenomena (Mao *et al*., 2017).

In the present study, the effect of vacuum level, treatment time, and $CaCl_2$ concentration was investigated for a new cultivar of black cherry tomato 'cv. OG' in Vietnam. The quality of fruits (in polyethylene (PE) and polypropylene (PP) bags) was evaluated after infiltration and during storage at 10-12°C.

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 in a garden house at Nam Long farm, Vinh Long province. Tomatoes were harvested 28 days after fruit formation. Fruits were packed into a perforated styrofoam box. They were transported to the Food Technology Laboratory of Can Tho University within 1 hr. Tomatoes were washed and then dipped into the water which was aerated with ozone for 15 min by a Z755 2-nozzle ozone generator, Vietnam (ozone-generating of 80.4 mg/h). Fruits were drained and used for all experiments.

Experimental design

Optimization of fruit treatment in calcium chloride solution by vacuum technique

This experiment was optimized using the Response Surface Methodology (RSM) with a model of Central Composite Design (CCD). The experiment was designed with three factors (Table 1). The total run was 20, including six replications at the center point. Each sample was 500 g of tomatoes and a ratio of raw material and $CaCl_2$ solution was 1:1. The infiltration process was carried out in vacuum equipment (Rocker 400, Laftech, Australia). A control sample was also conducted by soaking tomatoes in a 1.42% $CaCl_2$ solution for 20 min at the atmospheric pressure. The firmness of tomatoes was measured. The SEM image was taken for the cross-section of fruit slices.

Determination of shelf life of tomatoes treated in $CaCl_2$ solution by vacuum infiltration method and contained in two types of bags

After treatment in $CaCl_2$ solution by vacuum technique with optimal parameters, the tomatoes were packed in PE and PP bags and stored in a refrigerator at 10-12°C. Each sample was 1000 g of tomatoes. The bags had the same size of 20×30×0.015 cm and perforated proportion of 0.5% compared to their area. After every two days, the weight loss (%), the content of bioactive compounds such as anthocyanin (mgCE/100 g), lycopene (µg/g), vitamin C (mg/100 g), total phenolic content (mgGAE/100 g) with DPPH free radical scavenging activity (%) and acceptability of consumers were evaluated.

Analytical method

Scanning electron microscope (SEM) images

SEM images were taken at Advanced Laboratory, Can Tho University, Vietnam. The tomatoes were cut horizontally at the middle into 1 mm thick slices and photographed with a scanning electron microscope (JEOL JSM-7600F, USA).

Firmness of tomatoes

The firmness of tomatoes was determined by Rheo Tex (SD 700, Sun Science Co. LTD, Japan). A 1 cm – diameter cylindrical probe with a flat end was used in this case. The force required to press vertically into the middle of fruits for a 4 mm distance was measured and expressed in g/cm^2.

Table 1. Experimental layout

Run	Vacuum level (X_1) (mmHg)	Treatment time (X_2) (min)	$CaCl_2$ concentration (X_3) (%)	Run	Vacuum level (X_1) (mmHg)	Treatment time (X_2) (min)	$CaCl_2$ concentration (X_3) (%)
1	516	15	1	11	600	15	1
2	550	12	0.75	12	600	15	1
3	550	18	0.75	13	600	15	1
4	550	12	1.25	14	600	20	1
5	550	18	1.25	15	600	15	1.42
6	600	15	0.58	16	650	12	0.75
7	600	10	1	17	650	18	0.75
8	600	15	1	18	650	12	1.25
9	600	15	1	19	650	18	1.25
10	600	15	1	20	684	15	1

Weight loss

The weight loss (%) of fruits during storage was calculated according to equation 1, where W_o is the weight of tomatoes at the beginning (g); W_i is the weight of tomatoes at various times during storage (g).

$$X\,(\%) = \frac{W_o - W_i}{W_o} \times 100 \qquad (1)$$

Anthocyanin content

The anthocyanin content was determined by the pH differential method (Lee *et al*., 2005) with some modifications. Tomato puree (5 g) was filled to a volume of 50 mL 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 700 nm versus a blank of distilled water. The anthocyanin content was calculated as cyanidin-3-glucoside equivalent (equation 2), where A is $(A_{520nm}–A_{700nm})$pH 1.0 – $(A_{520nm}–A_{700nm})$pH 4.5, M is 449.2 g/mol for cyanidin-3-glucoside, k is the dilution factor, l is the pathlength (cm), ε is 26900 – molar extinction coefficient for cyanidin-3-glucoside ($L\times mol^{-1}\times cm^{-1}$), V is the volume of extract (mL), m is the weight of sample (g).

$$\underset{(\text{mgCE}/100\text{ g})}{Anthocyanin} = \frac{A\times M\times k\times V}{m\times\varepsilon\times l} \times 100 \times 1000 \qquad (2)$$

Lycopene content

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

$$Lycopene\ (\mu g/g) = \frac{A_{503}\times 31.2}{m} \qquad (3)$$

Vitamin C content

The vitamin C content was determined by the titration method (Lam *et al*., 2004). Tomato puree (10 g) was filled to a volume of 100 mL with 5% HCl solution and filtered through a filter paper. The filtrate (10 mL) was added 5 drops of the 1% starch solution and titrated with the 0.001N KIO_3/KI solution until the blue-black color appears. For the control, the sample extract was replaced by the 1% HCl solution. The vitamin C content was calculated using equation 4, where a and b is the volume of 0.001 N KIO_3/KI solution used for titration the extract and the control, respectively (mL), *100* is the volume of extract (mL), *0.088* is the weight of ascorbic acid corresponds to 1 mL of 0.001 N KIO_3/KI solution (mg), m is the weight of sample (g).

$$\underset{(\text{mg}/100\text{ g})}{Vitamin\ C} = \frac{(a–b)\times 0.088\times 100}{10} \times \frac{100}{m} \qquad (4)$$

Total phenolic content

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

$$Phenolic\ (\text{mgGAE}/100\text{ g}) = \frac{\text{C}\times\text{V}}{\text{m}} \times \text{k} \times 100 \qquad (5)$$

Antioxidant activity

Antioxidant activity was determined using the DPPH assay (Teixeira *et al*., 2013) with some modifications. Tomato puree (5 g) was filled to a volume of 50 mL with 95% ethanol and extracted for 60 min. The mixture was then separated by a centrifuge at 7000×g for 10 min. The supernatant (0.1 mL) was added 2 mL of DPPH solution (0.21 mM in 95% ethanol). For the control, the sample extract was replaced with 95% ethanol. The mixture was kept for 1 hr before absorbance reading at 517 nm. The percentage of DPPH free radical scavenging was calculated by equation 6, where $A_{control}$ is the absorbance of control, A_{sample} is the absorbance of sample.

$$DPPH\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \qquad (6)$$

Consumer acceptability

Acceptability of consumers was estimated using the odds ratio for the relationship between two binary ("yes or no") variables (Bland & Altman, 2000). Twenty participants were asked to assess the acceptability of tomatoes [Is this tomato acceptable? Yes {1 score} No {0 score}] (Garcia *et al*., 2009).

Data analysis

Data analyses was carried out using STATGRAPHICS Centurion XV (U.S.A.). The significance/non-significance of results was determined using the two-way ANOVA and Duncan test. The logistic regression model was applied to analyze the acceptability of consumers for tomatoes by storage time. The correlation model between acceptability and storage time was established (equation 7).

$$\text{Acceptability} = \exp(\eta)/(1+\exp(\eta)) \quad (7)$$

RESULTS AND DISCUSSION

Optimization of $CaCl_2$ treatment of black cherry tomatoes by vacuum infiltration method

The results in Table 2 showed that the effect of individual independent variables (X_1, X_2, X_3) and quadratic values (X_1^2, X_2^2, X_3^2) were significant ($p<0.05$) but the effect of interactivity (X_1X_2, X_1X_3, X_2X_3) was not significant ($p<0.05$) when participated in the model.

The relationship between firmness and independent variables was established (equation 8). The correlation between the estimated and the experimental firmness of tomatoes was satisfactory (Figure 1).

$$\text{Firmness (g/cm}^2) = -9929.64 + 28.5859X_1 + 150.182X_2 + 1875.15X_3 - 0.0234796^* X_1^2 + 0.0775X_1X_2 + 0.01X_1X_3 - 5.79997X_2^2 - 2.16667X_2X_3 - 842.813X_3^2 \quad (8)$$

Table 2. Analysis of variance for firmness

Source	Sum of Squares	Df	F-Ratio	*p*-value
X_1: Vacuum level	85464.1	1	336.47	0.0000
X_2: Treatment time	51352.5	1	202.18	0.0000
X_3: $CaCl_2$ concentration	22665.7	1	89.24	0.0002
X_1^2	49529.8	1	195.00	0.0000
X_1X_2	1081.13	1	4.26	0.0941
X_1X_3	0.125	1	0.00	0.9832
X_2^2	38175.8	1	150.30	0.0001
X_2X_3	21.125	1	0.08	0.7846
X_3^2	39886.5	1	157.03	0.0001

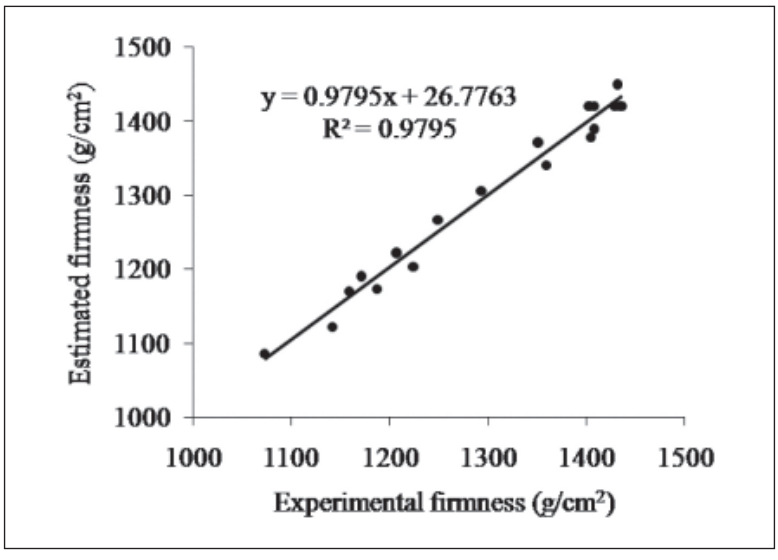

Fig. 1. Correlation between the experimentally determined values and the estimated values for the firmness of tomatoes.

Response surface graphs showed the interaction between vacuum level, treatment time, and $CaCl_2$ concentration to the firmness of black cherry tomatoes were presented in Figure 2. As the vacuum level, treatment time, and $CaCl_2$ concentration increased, the firmness of fruits also increased to 1477.81 g/cm^2, which tended not to increase continually. The model predicted that firmness reached the optimum value when tomatoes were infiltrated in a 1.09% $CaCl_2$ solution for 17 min at a vacuum level of 637 mmHg. As the content of calcium in tomato pericarp increases, it may strengthen cation bridges between uronic acid groups to create the stability of cell walls (Senevirathna & Daundasekera, 2010). Vacuum infiltration technology is used to replace the pressure infusion process, which helps to increase calcium content quickly contributing to improve fruit firmness (Senevirathna & Daundasekera, 2010).

The morphological features of the stomata of tomatoes based on their SEM images (Figure 3) demonstrated the effect of vacuum treatment on the texture of fruits. During vacuum treatment, gases in the food matrix expand and escape outside and after restoring atmospheric pressure, the pressure difference causes the liquid to penetrate the pores until the pressure reaches an inside and outside balance (Saurel, 2002).

Determination the shelf life of tomatoes treated in $CaCl_2$ solution at vacuum pressure and contained in two types of bag

The infiltration of tomatoes in $CaCl_2$ solution by vacuum method increased the firmness of fruit, therefore, the storage time at the temperature of 10-12°C was longer (34 days for the vacuum sample and 28 days for the control set in PE bags), compared to 30 days and 24 days in PP bags, respectively. During these periods, fruits were still undamaged. Calcium is the constituent of middle lamellae and the weakening of middle lamellae resulted in the softening of fruits during ripening (Bhattarai & Gautam, 2006). Calcium acts as a bridge between polygalacturonic acid molecules that strengthen the membrane, thereby reducing the rate of evaporation and respiration leading to slowing down the senescence (Bhattarai & Gautam, 2006). PE bags had better air permeability than PP bags (Dong, 2005), which helped to reduce the damage of fruits due to anaerobic respiration.

Weight loss

A progressive increase in weight loss was observed in all samples during storage. Evaporation and respiration are the main causes of the weight loss of fruit and vegetables, in which, the diffusion of vapor-phase is due to the difference in water vapor

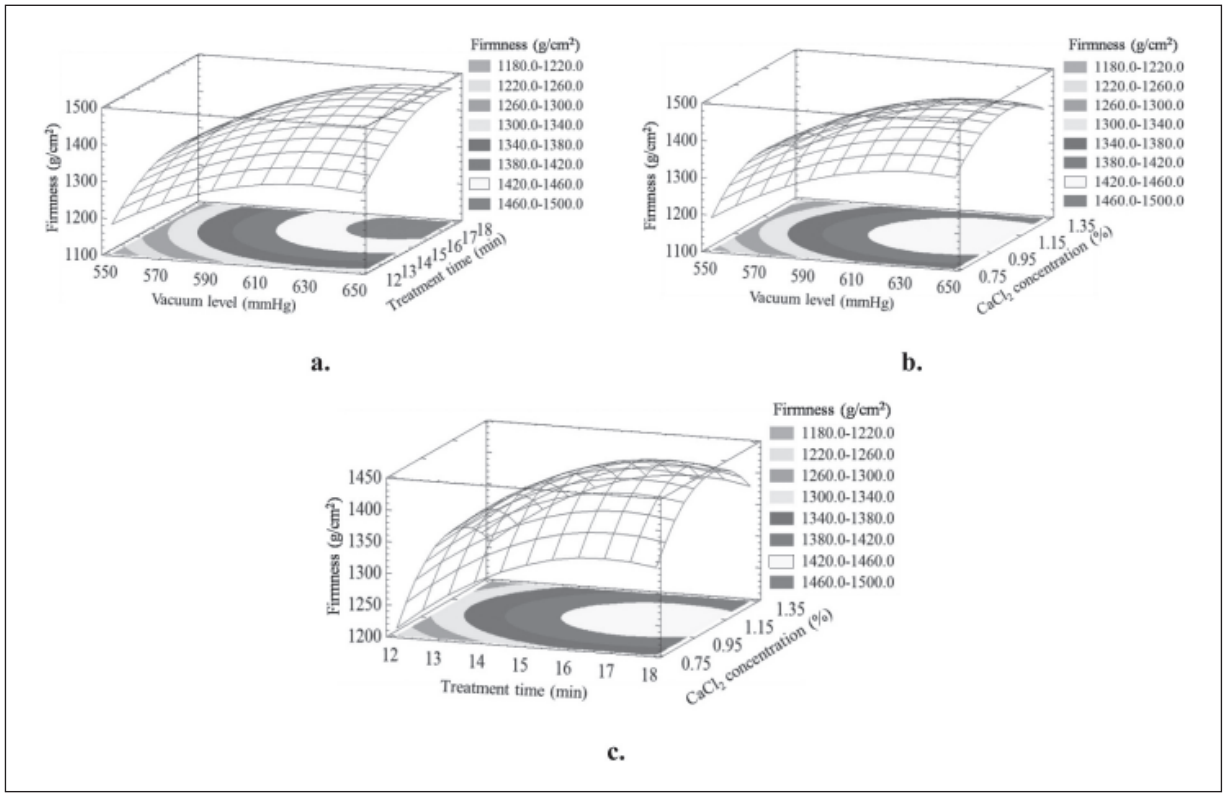

Fig. 2. The response surface and contour plots of firmness as the function of (**a**)Vacuum level and treatment time; (**b**) Vacuum level and $CaCl_2$ concentration; (**c**) Treatment time and $CaCl_2$ concentration.

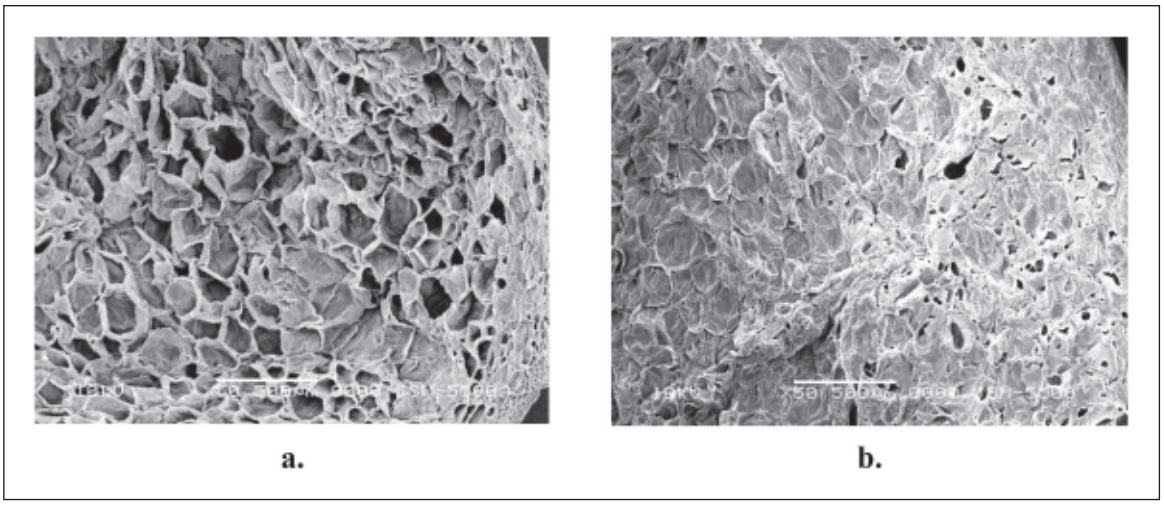

Fig. 3. SEM images of tomato fruits infiltrated in 1.09% $CaCl_2$ solution for 17 min (**a**) At atmospheric pressure; (**b**) At a vacuum level of 637 mmHg.

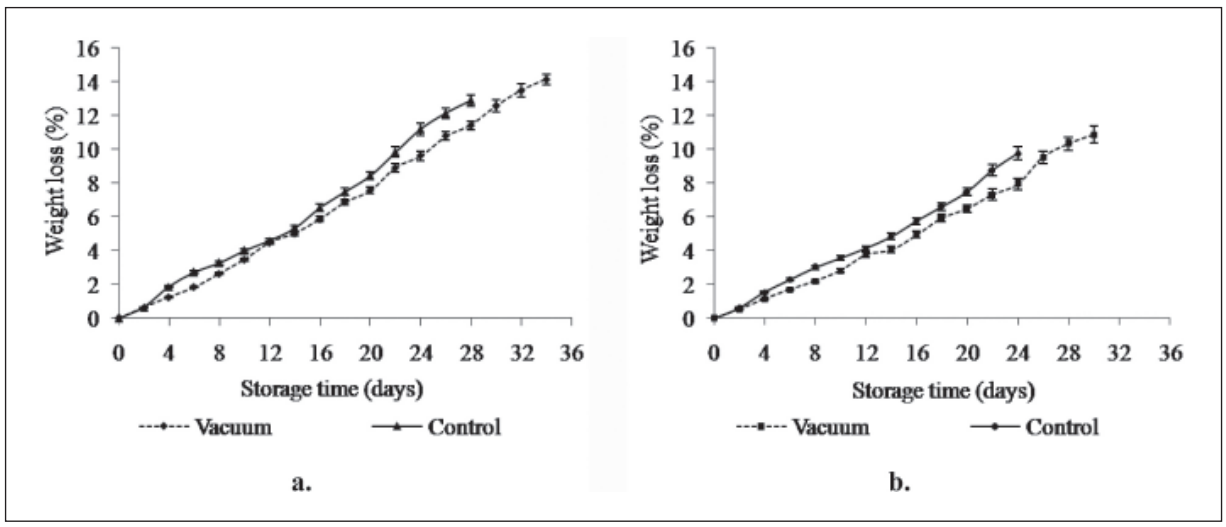

Fig. 4. Weight loss of black cherry tomatoes with/without vacuum treatment by storage time (**a**) PE bags; (**b**) PP bags.

pressure between material surface and environment (Pila *et al*., 2010). Fruits that were vacuum-treated in PE and PP bags had a corresponding weight loss of 9.62% and 7.94% after 24 storage days. These values for control samples were higher with 11.19% and 9.76%, respectively (Figure 4). Pila *et al*. (2010) reported that calcium enhances the structure of the membrane, thereby reducing the loss of protein and phospholipid components.

Vitamin C content

The vitamin C content (Figure 5) increased drastically in the early storage period. Tomato belongs to the group of climacteric fruits, therefore, after picking from the plant, the fruit continues to metabolize and ripen completely (Toor & Savage, 2006). The vitamin C content of fruits with vacuum treatment contained in PE and PP bags reached a maximum of 76.54 mg/100 g and 74.99 mg/100 g, which was higher than the control sample (73.85 mg/100 g in PE bags and 72.09 mg/100 g in PP bags). However, in the later stage of storage, the vitamin C content tended to decrease due to the ripening process of fruits stopped. In another study, where tomatoes were stored at 4°C, Galani *et al*. (2017) also showed a 71.8% decrease in vitamin C content after 14 days. During storage at low temperature, the fruits synthesize vitamin C as a response to stress, after that, oxidation by enzymes leads to the losses of vitamin C (Galani *et al*., 2017).

Lycopene content

The lycopene content was observed to increase significantly during storage (Figure 6). The ripening of tomato fruits retarded by the vacuum technique used for treatment in $CaCl_2$ solution, the lycopene

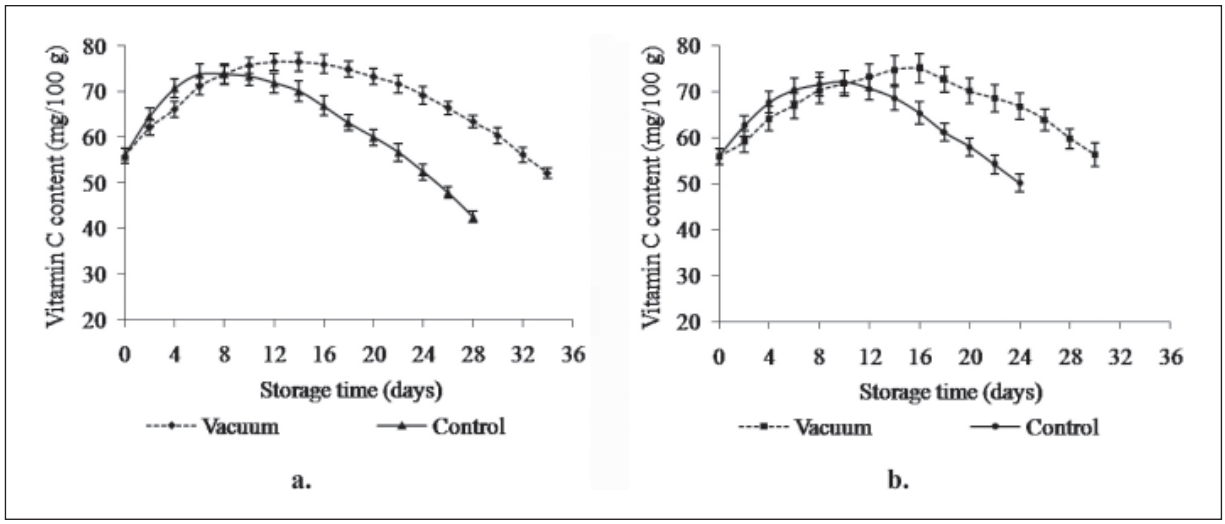

Fig. 5. Vitamin C content of black cherry tomatoes with/without vacuum treatment by storage time (**a**) PE bags; (**b**) PP bags.

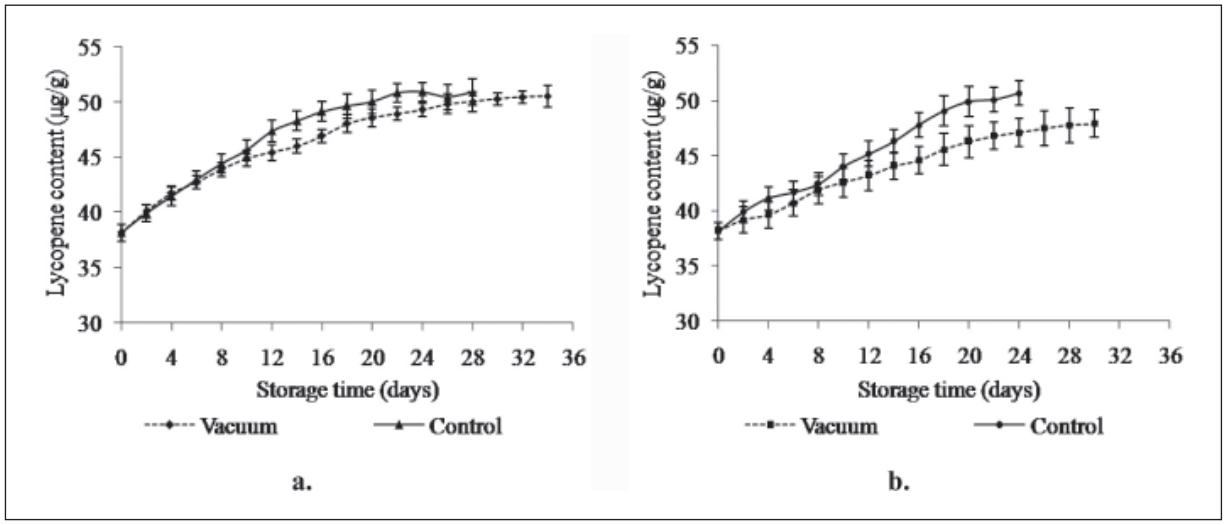

Fig. 6. Lycopene content of black cherry tomatoes with/without vacuum treatment by storage time (**a**) PE bags; (**b**) PP bags.

content of treated and control samples was 48.57 and 50.01 µg/g, respectively, after 20 days of storage in PE bags in comparison to the initial content 38.17 µg/g. The lycopene accumulation during ripening leads to an increase in the redness of tomatoes (Toor & Savage, 2006).

Anthocyanin content

Unlike lycopene, the anthocyanin content did not change significantly during storage (Figure 7) because anthocyanins were not synthesized continually after harvest. For fruits with infiltration in $CaCl_2$ solution by vacuum method, the anthocyanin content decreased more slowly than the control set (4.21 mgCE/100 g on the 34th day compared to 4.22 mgCE/100 g on the 28th day in PE bags) from the initial value of 4.37 mgCE/100 g. Enzymes such as peroxidases, glycosidases, and polyphenoloxidases may have caused anthocyanin degradation during storage (Galani *et al*., 2017).

Total phenolic content

The total phenolic content of black cherry tomatoes tended to decrease during storage (Figure 8) with the highest loss recorded in fruits that vacuum treated and contained in PE bag (from 43.13 mgGAE/100 g to 33.77 mgGAE/100 g after 34 days). These results were similar to the previous study (Galani *et al*., 2017), a decrease in total phenolic concentration was observed during tomatoes storage at 4°C for 15 days and the degradation of phenolic compounds may be due to the catalytic activity of polyphenol oxidases.

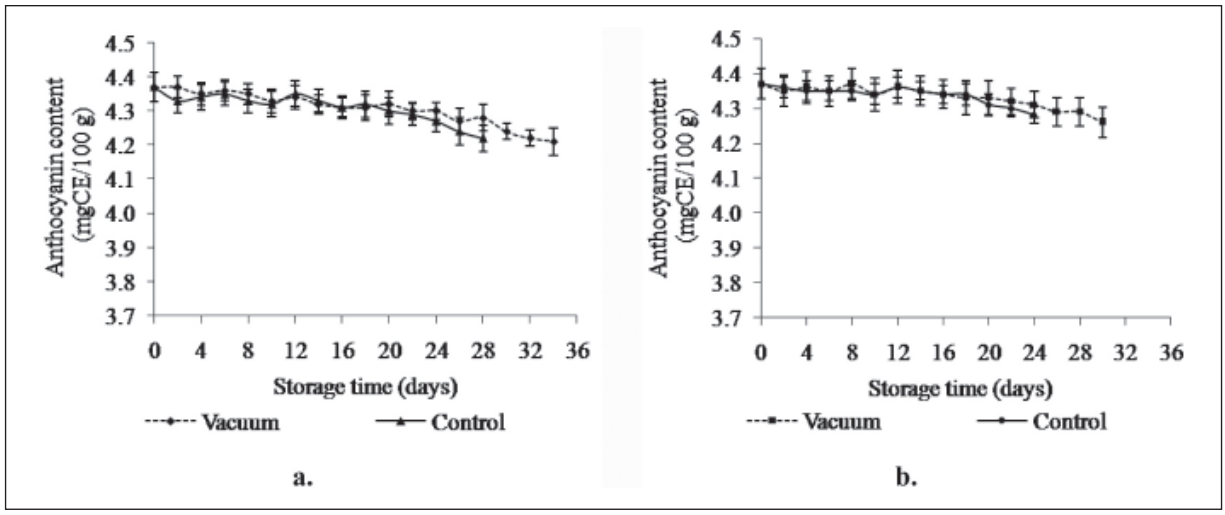

Fig. 7. Anthocyanin content of black cherry tomatoes with/without vacuum treatment by storage time (**a**) PE bags; (**b**) PP bags.

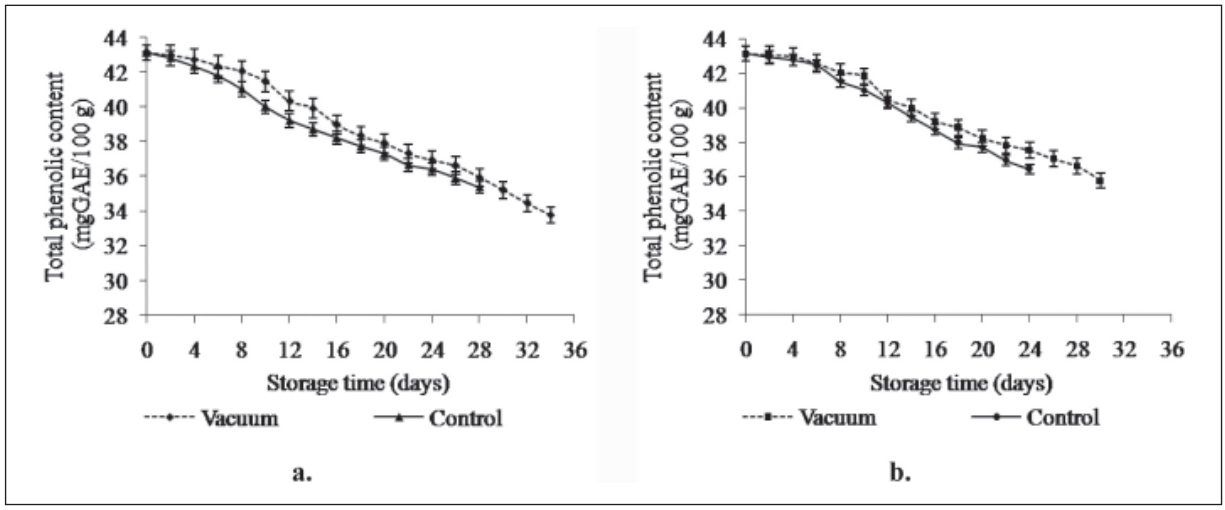

Fig. 8. Total phenolic content of black cherry tomatoes with/without vacuum treatment by storage time (**a**) PE bags; (**b**) PP bags.

DPPH free radical scavenging activity

Antioxidant activity of tomatoes was evaluated by the DPPH scavenging assay which tended to increase in the first days of storage and then dropped (Figure 9). Decrease of antioxidant activity during fruit storage may de due to the loss of vitamin C, anthocyanin, and phenolic compounds. Galani *et al*. (2017) also found a drastic decrease in antioxidant activity after storage tomatoes at 4°C for 15 days. However, the DPPH free radical scavenging of the vacuum treated fruits changed more slowly than the control sample.

The acceptability of consumers during storage

Results from the logistic regression model in Figure 10 showed that the acceptability of black cherry tomatoes maintained at high levels (>80%) for vacuum-treated samples when stored in PE and PP bags for 30 and 28 days, respectively. Meanwhile, the shelf-life of control samples was just only 22 and 20 days, respectively in PE and PP bags.

Results from Analysis of Deviance Table represented *p*-value for the model less than 0.05, it could be concluded that there was a statistically significant relationship between variables (the confidence level of 95%).

CONCLUSION

Implementing the pre-treatment process of black cherry tomatoes at the vacuum level of 637 mmHg, the treatment time of 17 min, and the $CaCl_2$ concentration of 1.09% that helped to achieve the good texture. The quality of fruits, treated with these

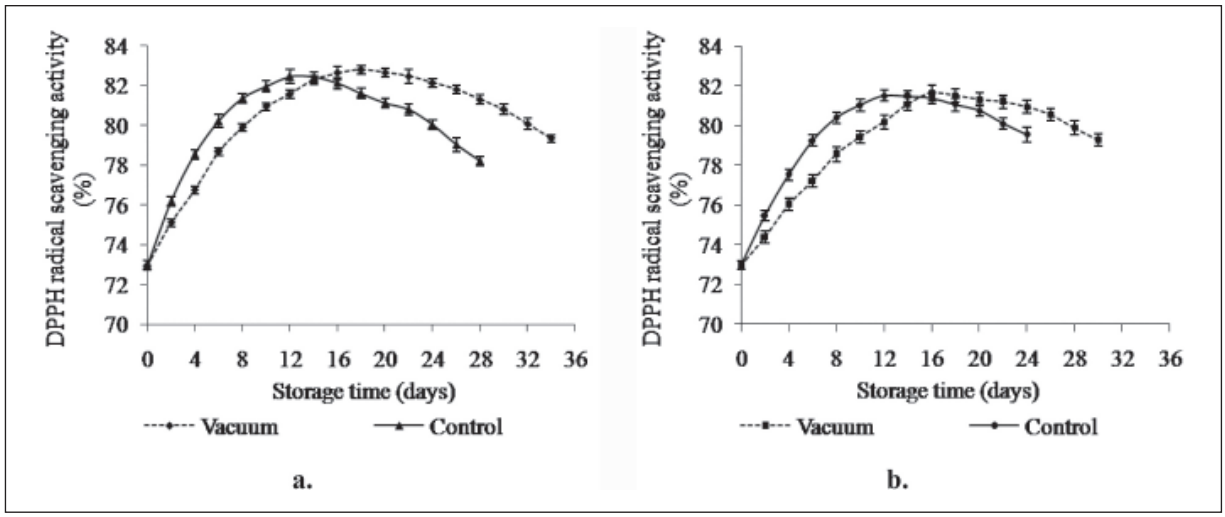

Fig. 9. DPPH free radical scavenging activity of black cherry tomatoes with/without vacuum treatment by storage time (**a**) PE bags (**b**) PP bags.

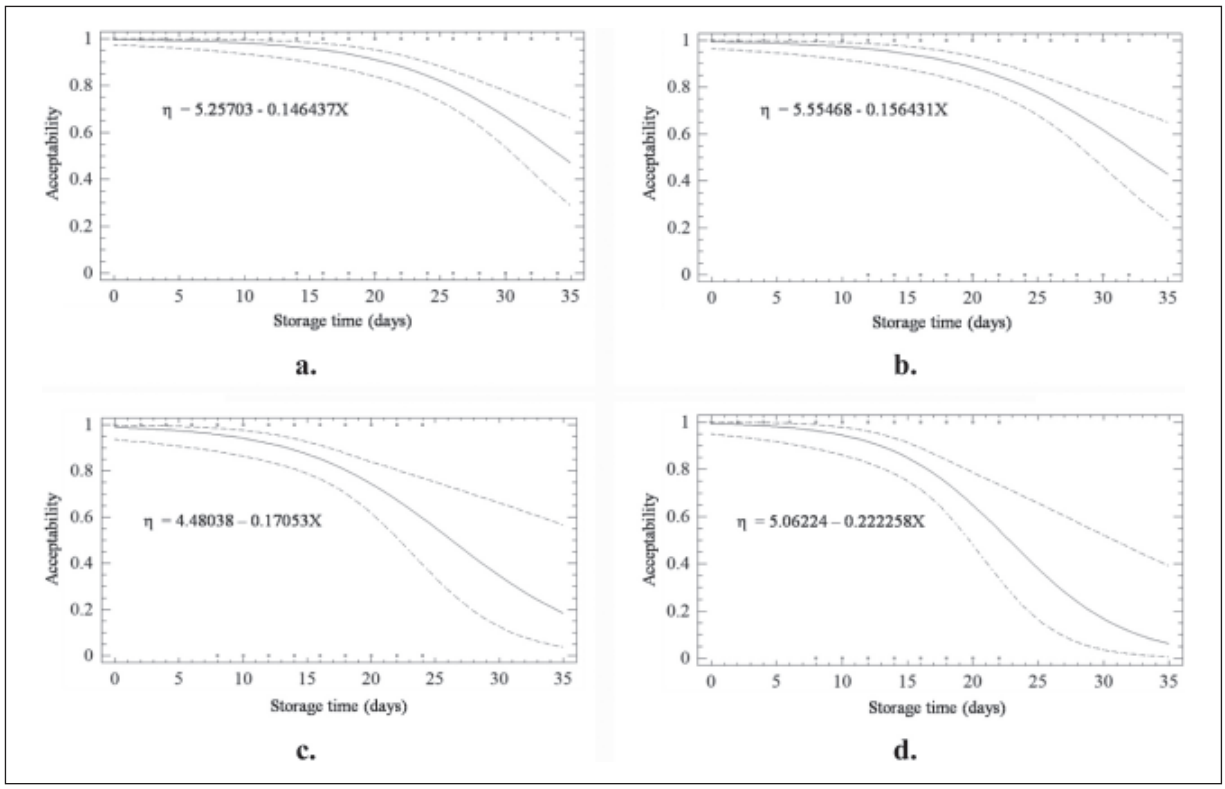

Fig. 10. Acceptability of black cherry tomatoes (**a**) Vacuum-treated sample in PE bag; (**b**) Vacuum-treated sample in PP bag; (**c**) Control sample in PE bag; (**d**) Control sample in PP bag.
Note: The band inside the two dashed lines is the 95% point-wise confidence interval; the solid line is a forecast.

optimal parameters changed more slowly during storage than the control samples in both PE and PP bags. Compared to the control samples (only 22 and 20 days stored in PE and PP bags, respectively), black cherry tomatoes that were treated in $CaCl_2$ solution by vacuum infiltration method and contained in PE and PP bags, then stored at 10-12°C had longer shelf life (30 and 28 days, respectively) and high acceptance to consumers. The vacuum technology was applied for black cherry tomato (*Solanum lycopersicum* cv. OG) treatment in $CaCl_2$ solution proved to increase the firmness and prolonged the storage time of fruits compared to the control samples.

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