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EFFECT OF OZONE TREATMENT ON THE QUALITY AND STORAGE TIME OF BLACK CHERRY TOMATOES

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ABSTRACT

This study was done to determine the effects of ozone treatment time (5-25 minutes) on the quality and shelf life of black cherry tomatoes (Solanum lycopersicum cv. OG). Results showed that the ozone treatment was carried out for 15 minutes (ozone-generating capacity of 80.4 mg/h, in a tank of 4 water liters, a sample weight of 500 g, a ratio 1:2 of tomatoes and water) helped to reduce total aerobic microorganisms and gave the best value for bioactive compounds content (anthocyanin 4.35 mgCE/100g, lycopene 37.01 µg/g, vitamin C 56.03 mg%, total phenolic (43.86 mgGAE/100g) and DPPH free radical scavenging activity (82.25%) and prolonged fruits storage time (14 days) compared to an untreated control sample (the mold appeared after only 3 days).

1. 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 dirty purplish-brown color (Mes, Boches, Myers & Durst 2008). In addition to the known bioactive compounds such as lycopene, vitamin C, purple tomatoes also contain anthocyanin (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). Tomatoes are easily perishable during harvesting, transportation and storage (Pila, Gol & Rao, 2010). If proper post-harvest technology 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). Ozone treatments are used to preserve quality during storage of commercially important fruits due to its ethylene oxidizing capacity and its antibacterial properties (Minas *et al*., 2014). In the present study, the effect of ozone treatment time was investigated for a new cultivar of black cherry tomato 'cv. OG' in Vietnam. The quality of fruits was evaluated after treatment and during storage in the PVC box at room temperature.

2. MATERIALS AND METHODS

2.1 Ozone treatment

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. All fruits with diseases and defects were removed. Fruits were packed into a perforated styrofoam box. They were transported to the Food Technology Laboratory of Can Tho University within three hours. Tomatoes were washed and then dipped into the water which was aerated ozone treatment for 5-25 mins by a Z755 2-nozzle ozone generator, Vietnam (ozone-generating of 80.4 mg/h, in a tank of 4 water liters, sample weight of 500 g, the ratio of material and water is 1: 2). Performing a control sample without ozone treatment. Fruits were drained and placed in PVC boxes (size of 13.5 cm x 12.0 cm x 9.5 cm, perforated rate of 1.6%) for storage at room temperature.

2.2 Analytical method

2.2.1 Weight loss

Weight loss (%) of fruits during storage was calculated according to the equation 1.

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

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).

2.2.2 Anthocyanin content

The anthocyanin content was determined by the pH differential method (Lee *et al.*, 2005) with some modifications. Tomato puree (5 g) was weighed and filled to a volume of 50 ml with the ethanol/water (1/1) solvent containing 1% HCl. The extraction process was carried out for 60 min at ambient temperature under subdued light by covering samples with aluminum foil. The mixture was then separated by a centrifuge (model Z323K, Hermle Labortechnik GmbH, Germany) at 7000×g for 10 min. The supernatant was diluted with two buffers of pH 1.0 and pH 4.5. The absorbance of diluted portions was read at both 520 and 700 nm using Spectrophotometer UV-VIS (722N, INESA, China) versus a blank with distilled water. The *anthocyanin content was calculated as cyanidin-3-glucoside equivalent (equation 2).*

$$Anthocyanin(\%) = \frac{A \times M \times k \times V}{m \times \varepsilon \times l} \times 100 \qquad (2)$$

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 dilution factor; l is pathlength (cm); ε is 26900 molar extinction coefficient for cyanidin-3-glucoside ($L \times mol^{-1} \times cm^{-1}$); V is the volumn of extract (ml), m is the weight of sample (g).

2.2.3 Lycopene content

The lycopene content was determined by the low volume hexane extraction method (Fish *et al.*, 2002; Davis *et al.*, 2003). Tomato puree (0.6 g) was weighed into a 40 ml vial with 5 ml of acetone containing 0.05% butylated hydroxytoluene, 5 ml of 95% ethanol and 10 ml of hexane. Sample was extracted for 15 min on a shaker (SK600, Lab Companion, Korea) at a speed of 180 rpm. Then add 3 ml of deionized water and continue the shaking for another 5 min. The vial was left for 5 min to allow the mixture to separate. The absorbance of the supernatant layer was read at 503 nm against a blank of hexane. The content of lycopene in samples was determined using equation 3.

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

Where A_{503} is absorbance of extract at 503 nm; m is the weight of sample (g).

2.2.4 Vitamin C content

The vitamin C content was determined by the titration method with iodine (Tran Bich Lam, Ton Nu Minh Nguyet & Dinh Tran Nhat Thu,

2004). Tomato puree (10 g) was weighed and filled to a volume of 100 ml with the 5% HCl solution. The mixture was shaken and filtered through a filter paper. The filtrate (10 ml) was taken into a 100 ml Erlenmeyer flask, added 5 drops of 1% starch solution and titrated with 0.001 N KIO_3/KI solution until the blue-black color of the starch - triiodide complex appears. In the control sample, the vitamin C extract was replaced by a 1% HCl solution. Vitamin C content in the sample was calculated using equation 4.

$$Vita\min C(mg\%) = \frac{(a-b)\times 0.088\times 100}{10}\times\frac{100}{m} \quad (4)$$

Where a is the volume of 0.001 N KIO_3/KI solution used for vitamin C extract (ml); b is the volume of 0.001 N KIO_3/KI solution used for control sample; 100 is the volume of volumetric flask (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).

2.2.5. Total phenolic content

The total phenolic content was determined using Folin-Ciocalteu reagent (Gougoulias *et al.*, 2012; Teixeira *et al*., 2013) with some modifications. Tomato puree (5 g) was weighed and filled to a volume of 50 ml with 95% ethanol. The extraction was carried out at ambient temperature for 60 min under subdued light by covering samples with aluminum foil. The mixture was then separated by a centrifuge at 7000×g for 10 min. Taking 0.2 ml of the supernatant into test tubes, adding 1.0 ml of 10% Folin-Ciocalteu, leaving for 5 min and then adding 1.2 ml of 5% Na_2CO_3. After 2 hrs, the absorbance reading was recorded at 750 nm. The calibration curve of gallic acid was prepared in the 0.01-0.1 mg/ml range. The total phenolic content was calculated as gallic acid **equivalent (equation 5).**

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

Where C is the content of gallic acid was derived from the standard curve (mg/ml); V is the volume of extract (ml); m is the weight of sample (g); k is dilution factor.

2.2.5 Antioxidant activity

Antioxidant activity was determined using the DPPH assay (Teixeira *et al*., 2013). Tomato puree (5 g) was weighed and filled to a volume of 50 ml with 95% ethanol. The extraction was carried out at ambient temperature for 60 min under subdued light by covering samples with aluminum foil. The mixture was then separated by a centrifuge at 7000×g for 10 min. Taking 0.1 ml of the supernatant into test tubes and adding 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 ***f***ree **r**adical **s**cavenging ***was calculated by equation 6***.

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

Where $A_{control}$ is absorbance of the control; A_{sample} is absorbance of the sample.

2.2.6 Total aerobic microorganisms

The total aerobic microorganisms were determined by counting colonies on PCA (Plate Count Agar) medium (Tran Linh Thuoc, 2003). Tomato puree (10 g) was weighed and added to a flask containing 90 mL SPW (Saline Pepton Water) that had been autoclaved. Shaking well for 2 minutes. The homogenous sample was further diluted in decimal ranges. The diluted sample (1 mL) was transferred to a sterile Petri dish, pouring into each dish 10-15 mL of PCA medium cooled to 45 °C, shaking to disperse the sample evenly into the medium, incubating

at 30 °C for 72 hours. The density of total aerobic microorganisms in 1 g sample was calculated by equation 7:

$$A(CFU/g) = \frac{N}{n_i V f_i + \cdots + n_i V f_i} \quad (7)$$

Where A is the number of bacterial cells in 1 g sample; N is the total number of colonies counted on dishes; n is the number of inoculated dishes at each dilution; V is the volume of sample inoculated in each dish (mL); f is the corresponding dilution.

2.3 Estimating the 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, Sriwattana, No, Corredor & Prinyawiwatkul, 2009).

2.4 Data analysis

The experiment was carried out with three replicates. Microsoft Excel software is used to calculate and draw graphs. Data analyses were carried out using STATGRAPHICS Centurion XV (U.S.A.). The significance/non-significance of results was determined using the One-Way ANOVA and Duncan test. The logistic regression model was applied to analyze the acceptability of consumers for tomatoes by storage time.

3. RESULTS AND DISCUSSIONS

3.1 Total aerobic microorganisms

Results showed that the total aerobic microorganisms of the untreated black cherry tomatoes were $4.5x10^4$ CFU/g. When samples were subjected to ozone treatment for 5 to 25 minutes, the aerobic microorganisms density decreased (**Table 1**). Among them, the aerobic microorganism densities of ozone treatment samples for 20 and 25 minutes was lowest and were not different significantly ($1.7x10^2$ and $1.6x10^2$ CFU/g, respectively). Similarly, after 10 and 15 minutes of ozone treatment, the total aerobic microorganisms were also had no significant difference ($2.2x10^2$ and $2.8x10^2$ CFU/g, respectively). Ozone can kill microorganisms, especially effective against gram-positive, gram-negative and yeasts (Restaino, Frampton, Hemphill & Palnikar, 1995). According to the Vietnam Ministry of Health (2007), the limit of total aerobic microorganisms on fresh vegetable only need to ensure Good Agricultural Practice (GAP).

Table 1. Effect of ozone treatment time on the total aerobic microorganisms

Ozone treatment time (minutes)	Total aerobic microorganisms (CFU/g)
Control	$4.5x10^{4*d}$
5	$2.8x10^{2c}$
10	$2.2x10^{2b}$
15	$2.1x10^{2b}$
20	$1.7x10^{2a}$
25	$1.6x10^{2a}$

*Notes: *Average value of three repetitions. The different letters that accompanied the average value in the same column showed the difference at the 5% significance level (P <0.05).*

3.3 The content of bioactive compounds

The ozone treatment had been shown to affect the content of bioactive compounds and the DPPH free radical scavenging ability of black cherry tomatoes (**Table 2**).

Table 2 Effect of ozone treatment time on the content of bioactive compounds and the DPPH free radical scavenging ability of black cherry tomatoes.

Table 2. Effect of ozone treatment time on the content of bioactive compounds and the DPPH free radical scavenging ability of black cherry tomatoes

Time (minutes)	Anthocyanin (mgCE/100g)	Lycopene (µg/g)	Vitamin C (mg%)	Total phenolic (mgGAE/100g)	DPPH (%)
Control	4.40*c	31.81a	48.85a	39.38a	73.59a
5	4.40c	32.43ab	51.10b	39.30a	74.19a
10	4.38c	38.70d	55.68c	40.46ab	79.38b
15	**4.35bc**	**37.01cd**	**56.03c**	**43.86d**	**82.25b**
20	4.32ab	35.96cd	49.69ab	43.48cd	79.60b
25	4.30a	34.69bc	47.93a	41.70bc	76.27a

*Notes: *Average value of three repetitions. The different letters that accompanied the average value in the same column showed the difference at the 5% significance level (P <0.05).*

Ozone treatment time affected the anthocyanin content of black cherry tomatoes. The untreated control sample had the highest anthocyanin content (4.40 mgCE/100g). When ozone treatment for 5 to 25 minutes, anthocyanin content tended to decrease (from 4.40 to 4.30 mgCE/100g). However, compared with the control sample, the anthocyanin content of fruits treated with ozone for 5 to 15 minutes had no significant difference. Peroxidase promotes anthocyanin oxidation by direct and indirect mechanisms (Barth, Zhou, Mercier & Payne, 1995). Increased peroxidase activity in plants is associated with ozone damage (Patton & Garraway, 1986).

Ozone treatment also influenced the lycopene content of black cherry tomatoes. The lycopene content of the treated samples at different times was higher than the untreated sample and reached the highest value when treated for 10 minutes (38.70 µg/g). However, there was no significant difference in lycopene content in treated samples for 10 to 20 minutes. This result was consistent with the research of Tzortzakis, Borland, Singleton and Barnes (2007) observed an increase in β-carotene, lutein and lycopene content in ozone-treated tomatoes.

The vitamin C content of black cherry tomatoes also increased after ozone treatment. Ozone treatment for 15 minutes yielded the most optimal effect (56.03 mg%). Meanwhile, the vitamin C content also reached a high value when treated for 10 minutes (55.68 mg%) and did not show a significant difference compared to the 15 minutes treated sample. Monaco, Costa, Uliana and Lima (2014) also studied mangoes and found that a 20-minute ozone treatment increased the content of vitamin C. Ozone is an oxidant and vitamin C is effective against oxidative stress (Davey *et al.*, 2000). The increase in vitamin C content after ozone treatment may also be due to the inhibitory effect of ozone on enzymes such as ascorbate peroxidase and ascorbate oxidase (responsible for ascorbic acid degradation), by contrast, a high levels of ozone can cause cell damage that

leads to a decrease in ascorbic acid by promoting ascorbate oxidase activity (Ali, Ong & Forney, 2014).

The total phenolic content of ozone-treated black cherry tomatoes for 5 and 10 minutes was not significantly different from the untreated sample. Meanwhile, all samples treated for a longer time (15 to 25 minutes) had higher total phenolic content than the control. In particular, the treatment time of 15 minutes gave the highest phenolic content (43.86 mgGAE/100g) but not significantly different from the time of 20 minutes (43.48 mgGAE/100g). Alothman, Kaur, Fazilah, Bhat and Karim (2010) also showed that the total phenolic and flavonoid content of pineapple and bananas increased significantly when exposed to ozone for up to 20 minutes with the simultaneous increase in FRAP and DPPH values. The increased phenolic content may be due to the activation of phenylalanine ammonia-lyase which is one of the main enzymes that participated in the synthesis of phenolic compounds in plant tissue. The increase in phenolic and flavonoid content may also be due to cell wall transformation that occurs during ozone exposure, which may increase the ability to extract and release some conjugated phenolic compounds in cell walls (Alothman *et al*., 2010). In contrast, in another study, when exposed to ozone, phenolic and other antioxidant compounds were able to react with free radicals (Moldau, 1998), so that the content of bioactive compounds (lycopene, vitamin C, phenolic) reduced when ozone treatment took place for a long time.

Similarly, DPPH free radical scavenging ability increased when performing ozone treatment and reached the highest value at 15 minutes (82.25%) due to the increase in the content of three components of lycopene, vitamin C and phenolics which all had antioxidant activity.

3.4 The change of tomatoes quality by storage time

Ozone treatments are used to preserve quality during storage of commercially important fruits due to its ethylene oxidizing capacity and its antibacterial properties (Minas *et al*., 2014). In the control sample without ozone treatment, mold started to appear after 3 days of storage and after 5 days it was unusable. When treated with ozone for 5 minutes, the tomatoes were stored well for 9 days. Two samples treated with ozone for 10 and 15 minutes had the best storage time (16 days). This result was similar to those of Zambre, Venkatesh and Shah (2010) showed that the shelf life of tomatoes prolonged by 12 days when stored at 15 °C due to the reduction in the number of microorganisms on the surface. However, when the ozone treatment time was increased to 20 and 25 minutes, the storage time decreased (14 and 13 days, respectively) because the high ozone levels could lead to epidermal damage (Palou, Crisosto, Smilanick, Adaskaveg & Zoffoli, 2002).

3.4.1 Weight loss

A progressive increase in weight loss was observed in all samples during storage **(Figure 1**). 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 pressure between material surface and environment (Pila *et al*., 2010). Fruit treated with ozone for 10 and 15 minutes had 12.87% and 12.18%, respectively of weight loss after 16 days of storage at room temperature.

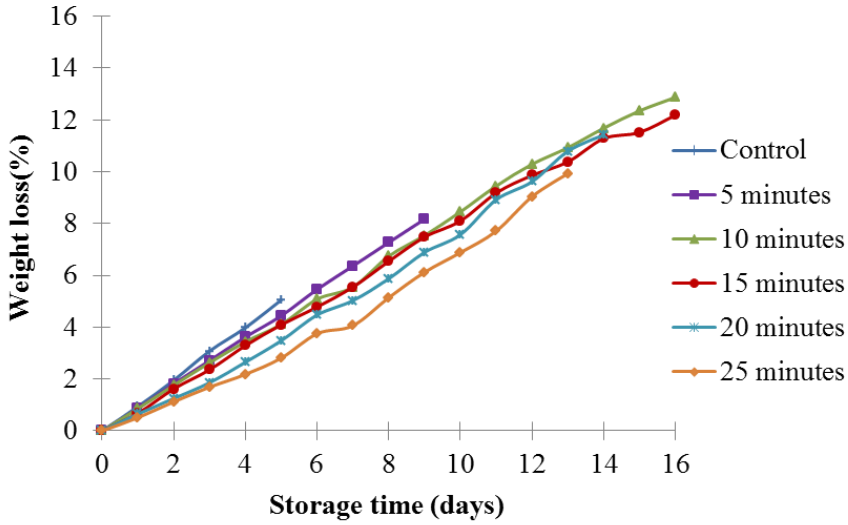


Figure 1. Weght loss of black cherry tomatoes by storage time when treated with ozone for different times

3.4.2 Lycopene content

The lycopene content was observed to increase significantly during storage (**Figure 2**). The ripening of tomato fruits retarded by the ozone technique. The lycopene content of ozone-treated samples for 10 and 15 minutes was 51.03 and 51.64 µg/g after 16 days of storage compared to the original content of 38.17 µg/g. The lycopene accumulation during ripening leads to an increase in the redness of tomatoes (Toor & Savage, 2006).

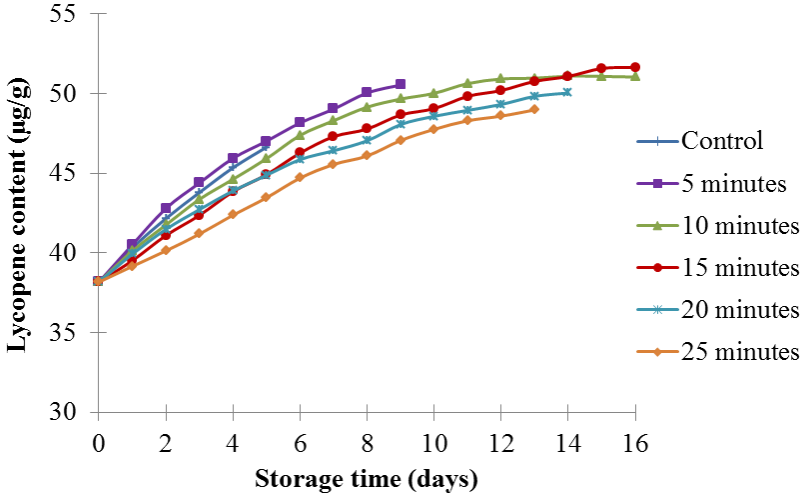


Figure 2. Lycopene content of black cherry tomatoes by storage time when treated with ozone for different times

3.4.3 Anthocyanin content

Unlike lycopene, the anthocyanin content did not change significantly during storage (**Figure 3**) because anthocyanins were not synthesized continually after harvest. Only two ozone-treated samples for 20 and 25 minutes had the anthocyanin content tended to slightly decrease in the last days of storage (anthocyanin content decreased from 4.37 mgCE/100g initially to 4.31 and 4.28 mgCE/100g, respectively after

13 days of storage). The fruits damage by ozone aeration for a relatively long time might have affected anthocyanin content. Enzymes such as peroxidases, glycosidases and polyphenol oxidases may have caused anthocyanin degradation during storage (Galani, Patel J.H, Patel N.J & Talati, 2017).

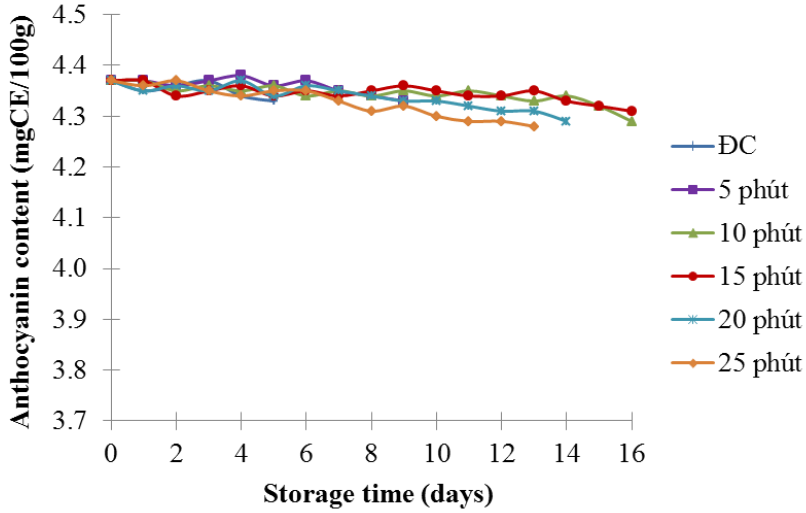


Figure 3. Anthocyanin content of black cherry tomatoes by storage time when treated with ozone for different times

3.4.4 Vitamin C content

The vitamin C content **(Figure 4)** 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). However, in the later stage of storage, the vitamin C content tended to decrease due to the ripening process of fruits stopped. Compared to the control sample without ozone treatment, the change of the vitamin C content of treated samples took place more slowly and the longer the treatment time, the slower the speed took place.

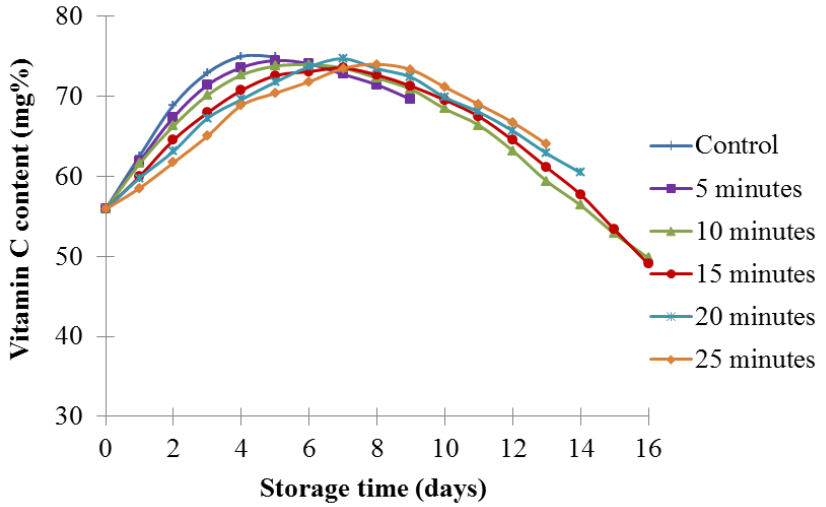


Figure 4. Vitamin C content of black cherry tomatoes by storage time when treated

with ozone for different times

3.4.5 Total phenolic content

The total phenolic content of black cherry tomatoes tended to decrease during storage (**Figure 5**). It was possible that during the fruits ripening there were changes (polymerization, oxidation and conjugation) of phenolic acids into states that could no longer be detected by spectroscopy, besides, the phenolic decrease was also related to the reduction of primary metabolism in ripening fruits, which led to a lack of substrates necessary for the biosynthesis of phenolic compounds (Gruz, Ayaz, Torun & Stmad, 2011).

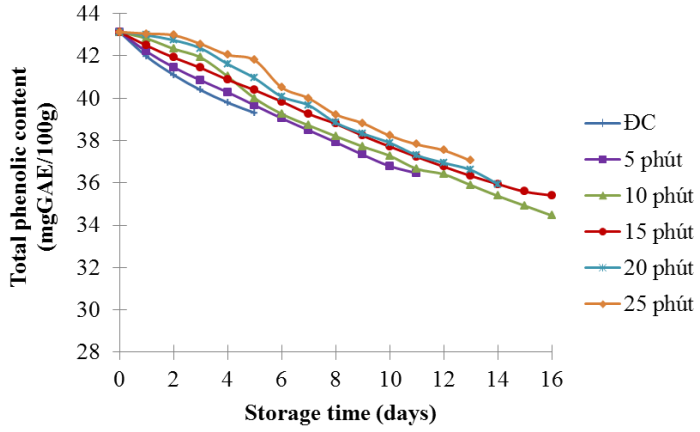


Figure 5. Total phenolic content of black cherry tomatoes by storage time when treated with ozone for different times

3.4.6 DPPH free radical scavenging ability

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 6**). During the ripening stage, the fruits constantly synthesized additional compounds such as vitamin C, lycopene, etc... these compounds belonged to the antioxidant group. At the end of the full ripening stage, the fruit began the aging stage, the antioxidant activity decreased due to the loss of vitamin C, anthocyanin and phenolic compounds.

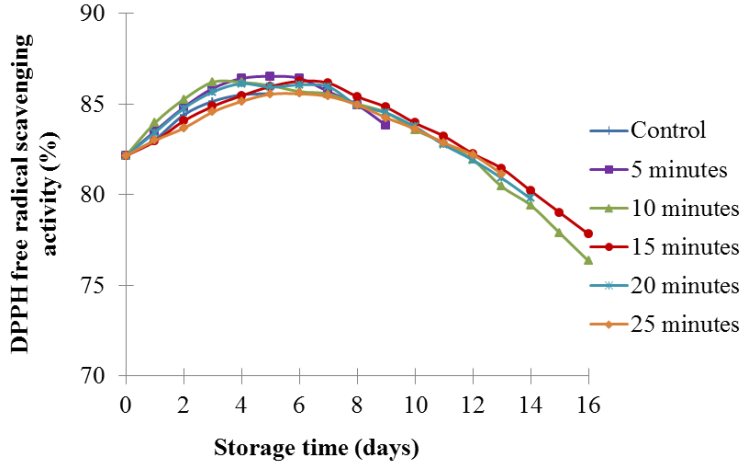


Figure 6. The DPPH free radical scavenging activity of black cherry tomatoes by storage time when treated with ozone for different times

3.4.7 The acceptability of consumers during storage

Sensory evaluation according to the possible method based on acceptance (score 1) or disapproval (score 0) of the assessor was carried out. The results were statistically calculated using a univariate method, the correlation model between acceptability and an independent variable (η) was established (equation 8).

$$Acceptability = \exp(\eta)/(1+\exp(\eta)) \quad (8)$$

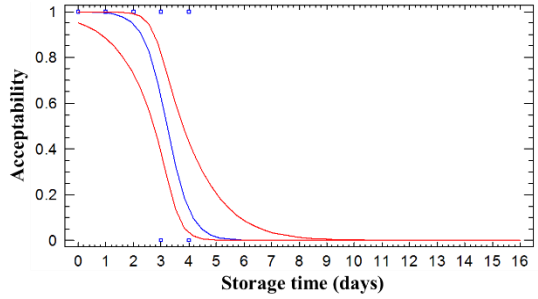
Where the relationship between the independent variable (η) and storage time of black cherry tomatoes was shown by the regression equations in **Table 3**.

Table 3. The regression equation described the relationship between acceptability and the independent variable (η) of ozone-treated samples with different times

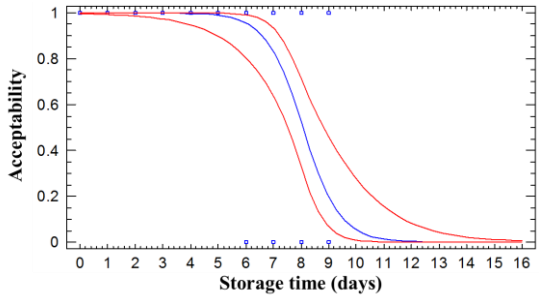
Sample	Logistic regression equation	P value (model)	P value (storage time)
Ozone-untreated control	η = 7,61297 - 2,36826X	0,0000	0,0000
Ozone-treated for 5 minutes	η = 12,0227 - 1,48988X	0,0000	0,0000
Ozone-treated for 10 minutes	η = 6,41356 - 0,407304X	0,0000	0,0000
Ozone-treated for 15 minutes	η = 6,09109 - 0,370959X	0,0000	0,0000
Ozone-treated for 20 minutes	η = 3,96203 - 0,250877X	0,0001	0,0001
Ozone-treated for 25 minutes	η = 4,36344 - 0,315591X	0,0000	0,0000

Note: η was an independent variable, X was the storage time (days)

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%). The results of the Likelihood test of the equations also gave a very small P-value for the storage time (P = 0.0000) showing the significant contribution of this factor to the models. From there, a correlation between product acceptability and storage time was established (**Figure 7**).



a.



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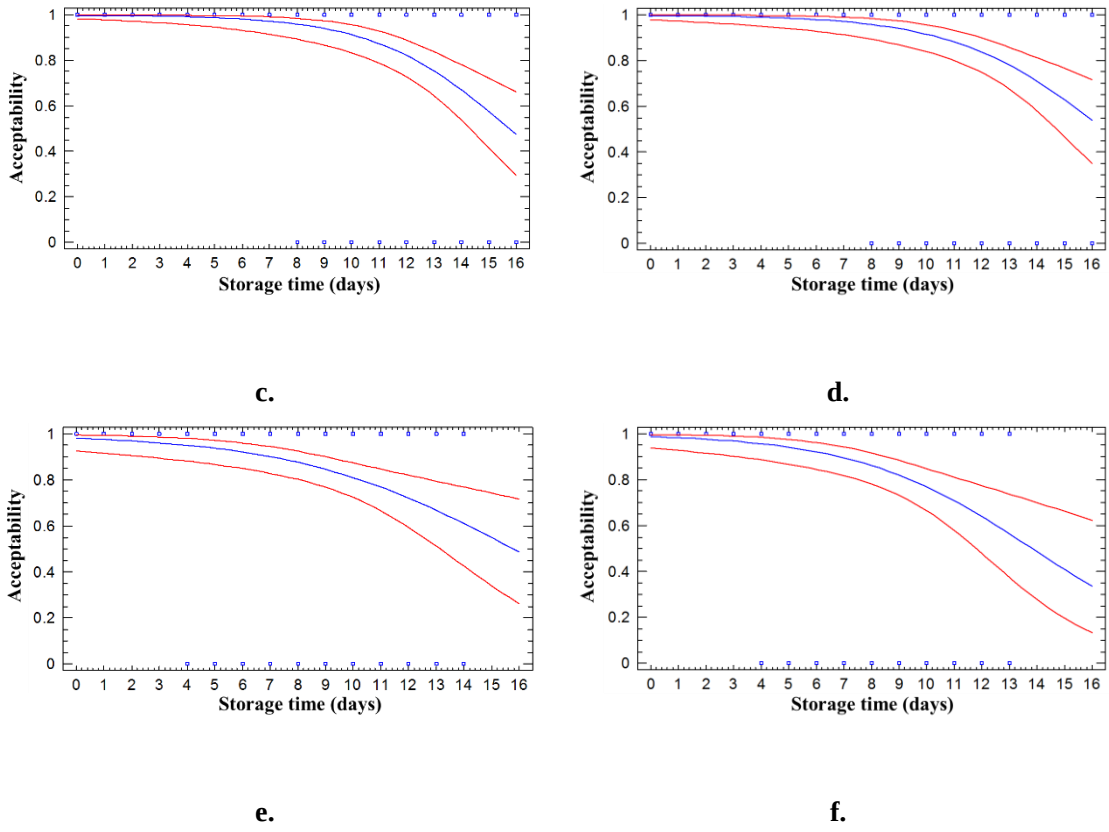

Figure 7. Acceptability of black cherry tomatoes by storage time when treated with ozone for different times

a. *Ozone-untreated control sample;* ***b.*** *Ozone-treated sample for 5 minutes*

c. *Ozone-treated sample for 10 minutes;* ***d.*** *Ozone-treated sample for 15 minutes*

e. *Ozone-treated sample for 20 minutes;* ***f.*** *Ozone-treated sample for 25 minutes*

Results from the logistic regression model showed that the acceptability of black cherry tomatoes maintained at high levels (more than 80%) for samples treated with ozone for 15 minutes for 14 days. Meanwhile, the acceptability of the control and ozone-treated samples for 5 and 10 minutes was only 3, 7 and 13 days, respectively. In contrast, when treated with ozone for a longer time (20 and 25 minutes), the acceptability of the tomatoes decreased, only 12 and 11 days, respectively.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

Ozone treatment had a positive impact on a significant reduction of microbial density. Accordingly, the content of bioactive compounds and the DPPH free radical scavenging ability, as well as the storage time of the black cherry tomatoes, were maintained at high levels when the fruits were ozone-treated for 15 minutes.

4.2 Recommendations

Studying the storage time of ozone-treated black cherry tomatoes at cool conditions in different types of packaging. Studying other methods of preserving fruits (treating with calcium chloride, ascorbic acid, citric acid, potassium sorbate, covering with biological compounds extracted from plants,...). Continuing further research on black cherry tomatoes such as processing some products (tomato sauce, dried tomatoes, etc...) to bring this material closer to consumers.

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