Postharvest Biology and Technology 171 (2021) 111363

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

# Synergistic inhibitory effect of 1-methylcyclopropene (1-MCP) and chlorine dioxide ($ClO_2$) treatment on chlorophyll degradation of green pepper fruit during storage

Yamin Du [a], Tong Jin [a], Handong Zhao [a], Cong Han [a], Fei Sun [a], Qingmin Chen [b,*], Fengli Yue [b], Zisheng Luo [c], Maorun Fu [a,*]

[a] College of Food Science and Engineering, Qilu University of Technology (Shandong Academy of Sciences), Jinan 250353, PR China
[b] College of Food Science and Engineering, Shandong Agriculture and Engineering University, Jinan 250100, PR China
[c] College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, PR China

ARTICLE INFO

*Keywords:*
Green pepper
1-Methylcyclopropene
Chlorine dioxide
Chlorophyll degradation
Chlorophyll degradation-associated genes
Gene expression

ABSTRACT

Degreening indicates the ripening and senescence process of green pepper fruit, which mainly results from chlorophyll degradation. To date, however, the effect of 1-Methylcyclopropene (1-MCP) alone or in combination with chlorine dioxide ($ClO_2$) on the chlorophyll degradation pathway of green pepper at the molecular level remains scarce. In this study, green peppers were treated with 1 μL $L^{-1}$ 1-MCP, 30 μL $L^{-1}$ $ClO_2$ alone and 1 μL $L^{-1}$ 1-MCP plus 30 μL $L^{-1}$ $ClO_2$, respectively and stored at 20 °C for 12 d. The results showed that 1-MCP + $ClO_2$ combination was superior in inhibiting color changes, decreasing the respiration rate, and exhibiting chlorophyll content as compared with 1-MCP or $ClO_2$ alone. Further study on the expression of genes related to chlorophyll degradation pathway revealed that pheophytinase (*PPH*), pheophorbide *a* oxygenase (*PAO*) and red chlorophyll catabolite reductase (*RCCR*) were suppressed by all treatments. The efficiency of the combined treatment (1-MCP + $ClO_2$) was better than that of 1-MCP or $ClO_2$ alone. While the expression of chlorophyllase (*CLH*) was not affected by $ClO_2$, but was significant suppressed by 1-MCP and 1-MCP + $ClO_2$. Therefore, our results indicate the different regulatory roles of 1-MCP and $ClO_2$ on the chlorophyll degradation pathway and provide an efficient method to preserve green pepper.

## 1. Introduction

Green peppers (*Capsicum annuum* L.) are appreciated by consumers for their high nutritional and functional value (Rodoni et al., 2015). However, the shelf life of green pepper is limited due to a series of physiological and biochemical changes after harvest, such as water loss, color changes and pathological deterioration (Finger and Pereira, 2016). Fruit appearance, especially color, is one of the main factors affecting customer acceptance. The change in color from green to red indicates the ripening process of the pepper fruit, and leads to lower market value owing to decay, weight loss, susceptibility to microbial infection and various other types of damage (Ilić et al., 2008; Wei et al., 2019). The degreening phenomenon is mainly due to the chlorophyll degradation (Roongruangsri et al., 2013).

Chlorophyll degradation is a multistep enzymatic process (Christ and Hörtensteiner, 2014). The first step in chlorophyll *a* degradation is the removal of the phytol tail. This reaction is catalyzed by chlorophyllase (CLH), producing chlorophyllide *a* (Matile et al., 1999). Then the central $Mg^{2+}$ of the chlorophyllide is removed by Mg-dechelatase to produce pheophytin *a* (Li et al., 2017). Under the catalysis of pheophytinase (PPH), pheophytin *a* is converted to pheophorbide *a* (Schelbert et al., 2009). Pheophorbide *a* is then transformed to a primary fluorescent chlorophyll catabolite (pFCC) by pheophorbide *a* oxygenase (PAO) and red chlorophyll catabolite reductase (RCCR) (Pružinská et al., 2007). Finally, the pFCC is transformed into non-fluorescent chlorophyll catabolites (NCCs) (Hauenstein et al., 2016). Chlorophyll *b*, however, is degraded only after conversion to chlorophyll *a*, and this process is executed by two chlorophyll *b* reductase compounds and a hydroxymethyl chlorophyll *a* reductase. Previous studies have reported that two chlorophyll *b* reductase compounds, non-yellow coloring 1 (NYC1) and NYC1-like (NOL) participated in the reduction of chlorophyll *b* to 7-hydroxymethyl chlorophyll *a*, and 7-hydroxymethyl chlorophyll *a* is

* Corresponding authors.
*E-mail addresses:* chenqingmin1@126.com (Q. Chen), skyfmr@163.com (M. Fu).

https://doi.org/10.1016/j.postharvbio.2020.111363
Received 24 June 2020; Received in revised form 15 September 2020; Accepted 18 September 2020
Available online 1 October 2020

converted to chlorophyll *a* by 7-hydroxymethyl chlorophyll *a* reductase (HCAR) (Rüdiger, 2002; Jibran et al., 2015; Meguro et al., 2011).

1-Methylcyclopropene (1-MCP) has been widely used in fruits and vegetables. Current studies on pears, apples and broccolis showed that 1-MCP can retard chlorophyll degradation by inhibiting chlorophyllase activity and reducing the expression of chlorophyll degradation associated genes (Cheng et al., 2012; Gómez-Lobato et al., 2012; Lv et al., 2020). However, as a side effect of 1-MCP, its application increased decay incidence in tomatoes (Estiarte et al., 2016), pear (Itai et al., 2012), apples (Janisiewicz et al., 2003), strawberries (Bower et al., 2003), and oranges (Porat et al., 1999). In pepper fruit, although some studies have shown that 1-MCP can be beneficial for maintaining market quality and extending shelf life of pepper fruits, Fernández-Trujillo et al. (Fernández-Trujillo et al., 2009) found 1-MCP increased the gray mold rot of red sweet pepper during storage in the domestic refrigerator. Ilić et al. reported that green bell pepper fruit treated with higher dose 1-MCP slightly increased the decay development (Ilić et al., 2012). Therefore, sterilization treatment is necessary for pepper storage besides 1-MCP treatment.

Chlorine dioxide ($ClO_2$) is a class A1 safe and effective disinfectant, which can control pathogenic microorganisms (Zhang and Fu, 2018). It is also suitable for fruits and vegetables to maintain their postharvest quality and extend shelf life (Yan et al., 2019). Only a few published studies have analyzed the influence of $ClO_2$ on pepper fruit. Du et al. (2007) reported that $ClO_2$ treatment significantly alleviated the respiration and decay of bell pepper during storage. In addition, $ClO_2$ treatment also maintained the ascorbic acid and chlorophyll content of the green bell pepper. Wei et al. (2019) studied the effect of $ClO_2$ treatment on color variation in long green pepper after harvest, and found that $ClO_2$ delayed reddening by suppressing the expression of genes related to the chlorophyll breakdown and carotenoid synthesis pathways.

Although several studies have reported that 1-MCP or $ClO_2$ treatments can maintain the postharvest quality and slow down the degradation of chlorophyll of pepper fruit. However, to date, the effect of 1-MCP alone or in combination with $ClO_2$ on the chlorophyll degradation pathway at the molecular level during storage have not been determined. Moreover, comparison of the efficiency of 1-MCP, $ClO_2$ and 1-MCP + $ClO_2$ treatments is missing. Therefore, the present study aimed to systematically evaluate the efficiency of 1-MCP, $ClO_2$ and 1-MCP + $ClO_2$ for maintaining the quality of postharvest green pepper and analyze the regulation of genes involved in chlorophyll degradation by different treatments. This research will reveal the regulatory role of different treatments in the chlorophyll degradation pathway and provide an efficient method to preserve green pepper fruit.

## 2. Material and methods

### 2.1. Plant material and treatments

Green peppers (*Capsicum annum* L. cultivar *Dangjo,* Korea) were harvested at green mature stage (25 d after flowering) in Shouguang City, China, and transported to the laboratory immediately. The initial firmness of green pepper was 3.47 ± 0.95 N, and the total soluble solid content was 4.6 ± 0.95 %. 1-MCP was purchased from Shandong Yingyangyuan Food Technology Co., Ltd, China. The solid $ClO_2$ releasing agent was self-made as described before (Yang et al., 2018). Pepper fruit with similar size, color and no visual defect were selected. The selected fruit were soaked with 0.5 % NaClO for 2 min before treatment to reduce microbial load. All fruit were randomly divided into four groups with 300 fruit in each group. According to the results of our previous publications (Wei et al., 2019; Zhang et al., 2019) and preliminary experiments (Figs. S.1), 1 μL $L^{-1}$ 1-MCP and 30 μL $L^{-1}$ ClO2 were selected as the appropriate treatment concentration. Four groups were treated as follows: control (no treatment), 1-MCP treatment (1 μL $L^{-1}$ final released gas concentration), $ClO_2$ treatment (1 g $ClO_2$ tablets could release about 30 μL $L^{-1}$ $ClO_2$ gas) and 1-MCP + $ClO_2$ treatment (combination of 1 μL $L^{-1}$ 1-MCP and 30 μL $L^{-1}$ $ClO_2$). All fruit were stored at 20 °C and 85 ± 5 % RH. Groups of 20 fruit each were stored in a 10 L plastic basket sealed with polyethylene storage bags (0.03 mm thickness, the permeability of $O_2$ and $CO_2$ were 7000−20000 $cm^3$ $(m^2*24\ h*atm)^{-1}$ and ≥50,000 $cm^3$ $(m^2*24\ h*atm)^{-1}$, respectively). After 24 h treatment, the polyethylene storage bags of all samples were punctured with two holes in the middle with a diameter of 6 mm in order to inhibit the anaerobic respiration of green peppers. Fruit were taken at 0, 3, 6, 9, and 12 d after treatment for further analysis. At each sampling point, three bags were taken out from each group. Fifteen fruit were randomly taken from each group to measure the quality attributes. All experiments were performed in triplicate.

### 2.2. Determinations of respiration rate, weight loss, firmness, total soluble solid (TSS), and ascorbic acid

The respiration rate was determined according to the method of Wei et al. (Wei et al., 2019). In brief, 15 green pepper fruit were placed in a 10 L sealed container at 20 °C for 1 h. The result was detected using gas analyzer (F-920, Felix, USA) and expressed as mg $CO_2$ $kg^{-1}$ $h^{-1}$.

Stored green peppers from each treatment were weighed at the beginning of the experiment and at 3 days interval during storage. Weight loss was expressed as the percentage loss of the initial weight.

Pepper fruit firmness was measured by texture analyzer (Universal TA, Shanghai Tengba Instrument Technology Co., Ltd, China). Each treatment contained three replicates with 15 peppers per replicate. The result was expressed as N.

Total soluble solid content (TSS) in pepper juice was determined by a refractometer (PAL-1, Atago, Tokyo, Japan) and the result was expressed as percentage. For each treatment, and replicate, 15 green pepper fruit were chopped and mixed together. Fifty-gram samples were used for preparing pepper juice. The rest was used for other quality attributes.

For ascorbic acid content determination, 5 g samples from 15 green pepper fruit per replicate were homogenized in 10 g $L^{-1}$ oxalic acid solution and made up to 100 mL, 10 mL supernatant was titrated with 2,6-dichlorophenol indophenol, until a persistent rose pink color has been obtained. The result was expressed as g $kg^{-1}$.

### 2.3. Measurements of surface color and chlorophyll content

The evaluation of color change included reddening index and color properties ($L^*$, $a^*$).

The reddening index was assessed by measuring the extent of reddening area on the surface of green pepper fruit using the following scale: 0 = no red on the fruit surface; 1= < 25 % fruit surface appeared red; 2 = 25 %-50 % fruit surface appeared red; 3 = 50 %-75 % fruit surface appeared red; 4 = > 75 % fruit surface appeared red. Reddening index was calculated using the formula. Each treatment contained three replicates with 15 peppers per replicate to evaluate the average values. Reddening index=Σ(reddening level x fruit number of this level)/(highest reddening level x total fruit number).

The change in peel color was determining using an X-Rite 528 spectrodensitometer (X-Rite Co. Ltd., Michigan, USA). Each fruit was measured at three points around the equator of the fruit. For each treatment, and replicate, 15 green pepper fruit were used. Color was recorded using the CIE $L^*$ (lightness) and $a^*$ (red/green) scale.

Chlorophyll content was determined using the method described by Wang et al. (2016). One-gram samples from 15 peppers were ground in 80 % (v/v) acetone, after the mixture was stood at 4 °C for 15 min, a spectrophotometer (V-1100D, Mapada Inc., Shanghai, China) was used to measure the absorbance of supernatant at wavelengths of 645 nm and 663 nm.

### 2.4. RNA isolation and quantitative reverse transcription-PCR (qRT-PCR) analysis

Total RNA was isolated from 0.05 g samples of 15 peppers, using the TaKaRa MiniBEST Plant RNA Extraction Kit (Takara Bio, Shiga, Japan) according to the manufacturer's instructions. RNA quality and concentration were evaluated with an MD2000 spectrophotometer (BioFuture, Britain). First-strand cDNA was synthesized using Primescript™ II 1st Strand cDNA Synthesis Kit (Takara Bio Inc.) following the manufacturer's recommendations. qRT-PCR was conducted with gene-specific primers (Table 1), SYBR® Premix Ex Taq™ (Takara Bio Inc.) and FQD-96A (Bioer, China). The *Ubi-3* gene was used as the internal control gene. Target gene expression levels were assessed by the $2^{-\Delta\Delta Ct}$ relative quantification method (Livak and Schmittgen, 2001). All experiments were repeated 3 times.

### 2.5. Statistical analysis

Completely randomized design with three replicates was used for all experiments. The result was given as the means with standard deviations (SD). Data were analyzed through one-way ANOVA and Duncan's multiple range test (SPSS version 20.0, Chicago, IL, U.S.A.). Differences were considered statistically significant when $P < 0.05$.

## 3. Results

### 3.1. Alteration of respiration rate, weight loss, firmness, total soluble solid (TSS), and ascorbic acid after different treatments

Respiration rate is an important indicator of physiological activity. As shown in Fig. 1A, regardless of the control or the treatment groups, the respiration rate of green pepper gradually increased, reached the highest value on the 6th day of storage, and then decreased. Compared with each treatment group, the control group had the highest respiration rate during the whole storage period. However, at the end of storage, significant influence was only observed between the control group and 1-MCP + $ClO_2$ treatment group ($P < 0.05$). This result indicated that the combination of 1-MCP and $ClO_2$ was the most effective treatment to reduce the respiration rate of green pepper, whereas 1-MCP or $ClO_2$ alone could only work within 9 d after treatment, but had no obvious effect at the end of storage.

The process of transpiration and respiration may cause water loss and reduce fruit weight. The changes in weight of green pepper during storage were shown in Fig. 1B. The weight loss continuously increased during storage. Compared with the control group, all treatments were effective in reducing weight loss ($P < 0.05$). After 12 d of storage, green peppers treated with 1-MCP + $ClO_2$ showed the lowest weight loss. However, no significant differences were found among 1-MCP, $ClO_2$ and 1-MCP + $ClO_2$ group.

There is a strong correlation between firmness and weight loss in bell pepper fruit (Maalekuu et al., 2004). With the extension of storage time, the firmness of green pepper decreased (Fig. 1C). Pepper fruit treated with 1-MCP + $ClO_2$ maintained the highest firmness during the storage. Both 1-MCP and $ClO_2$ had a good effect on firmness retention, and no significant difference was observed between 1-MCP and $ClO_2$ treatment ($P > 0.05$). This is consistent with the result obtained in weight loss.

Fig. 1D displayed the changes in TSS content. During the storage of green pepper, the hydrolysis of starch and other macromolecular substances resulted in the increase of TSS. Subsequently, TSS was consumed by respiration, and TSS content decreased. In the control group, the maximum value of TSS appeared on day 6, while in all treatment groups, the maximum value appeared on day 9. At the end of storage, significant differences were observed between the control group and the treatment groups ($P < 0.05$). The results showed that 1-MCP, $ClO_2$ and 1-MCP + $ClO_2$ treatment had remarkable effects on delaying the decrease of TSS content and 1-MCP + $ClO_2$ maintained the highest TSS content after the storage.

Ascorbic acid content showed a decreasing trend (Fig. 1E). The ascorbic acid content of green pepper in the control group was at the lowest level throughout the storage period. 1-MCP treatment was more effective than $ClO_2$ in reducing the loss of ascorbic acid content. Although 1-MCP + $ClO_2$ was the most effective treatment to maintain ascorbic acid content, no significant difference was observed between 1-MCP and 1-MCP + $ClO_2$ group ($P > 0.05$).

### 3.2. Effect of different treatments on color and chlorophyll change

In many fruits, including green pepper, there is a significate change in color during postharvest storage. The loss of green color is a valuable guide to evaluate pepper fruit quality. As shown in Fig. 2, the color change progress of green pepper fruit was extended after treatment with 1-MCP, $ClO_2$ and the combination of 1-MCP and $ClO_2$. The control group had the highest reddening index during the whole storage period and significant difference was observed between the control group and the treatment groups ($P < 0.05$). After 12 d of storage, the reddening index of the fruit treated with 1-MCP, $ClO_2$ and the combination was 52.8 %, 56.8 % and 41.2 %, respectively, while the untreated fruit was as high as 83.1 % (Fig. 3A). This indicated that all treatments could delay the reddening of green pepper and the combination of 1-MCP and $ClO_2$ had the best effect.

To further evaluate the color development, $a^*$ and $L^*$ value were measured (Fig. 3B and C). The increase of $a^*$ value represents a change in color from green to red. The $a^*$ value showed significant difference between the control group and the treatment groups ($P < 0.05$), and the $a^*$ value of the control group increased rapidly after the initial 3 d, reaching the highest value of 17.6 at the end of storage, while the treatment groups exhibited lower values. This is consistent with the reddening index. $L^*$ value (lightness) decreased from 49.3–30.8 in the control group, whereas the treatment groups remained higher values of 41.3 (1-MCP + $ClO_2$), 39.3 (1-MCP) and 35.5 ($ClO_2$), respectively.

The loss of green color is related to the decreased of chlorophyll content (Roongruangsri et al., 2013). The changes in chlorophyll content during storage are illustrated in Fig. 3D. The chlorophyll level decreased continuously. There was significant difference between the control group and the treatment groups during the whole storage period ($P < 0.05$), which indicates the greener color phenotype in different treatment groups. The final chlorophyll content of green pepper was 0.021 g $kg^{-1}$ in the control group, 0.065 g $kg^{-1}$ in the 1-MCP treatment group, 0.057 g $kg^{-1}$ in the $ClO_2$ treatment group, and 0.074 g $kg^{-1}$ in the combination treatment group. This demonstrated that pepper fruit treated with 1-MCP, $ClO_2$ and 1-MCP + $ClO_2$ could retard the breakdown of chlorophyll.

**Table 1**
Gene-specific primers for quantitative PCR analysis (5′-to-3′).

| Gene | Accession No. | Forward Primer | Reverse Primer |
|---|---|---|---|
| *CaCLH* | EU294210.1 | TCCACCAGCGGTTCTCAC | TCCCTCCAACAAATCTCCTCAT |
| *CaPPH* | XM_016703978 | AAGACCAGTGTTAGGGAAAG | GAGAAGGGCGAAAGTGT |
| *CaPAO* | KC176709.1 | CACCTCCAACAACACCAC | CCACTGAGACCCAGATTTA |
| *CaRCCR* | KC176711.1 | ATTCACTCCCTTCTTCCC | ATATTCAACGCTCCACCT |
| *Ubi-3* | AY486137.1 | TTGGCAAGCAACAATCAT | GCAGATGGACAGCAGGAC |

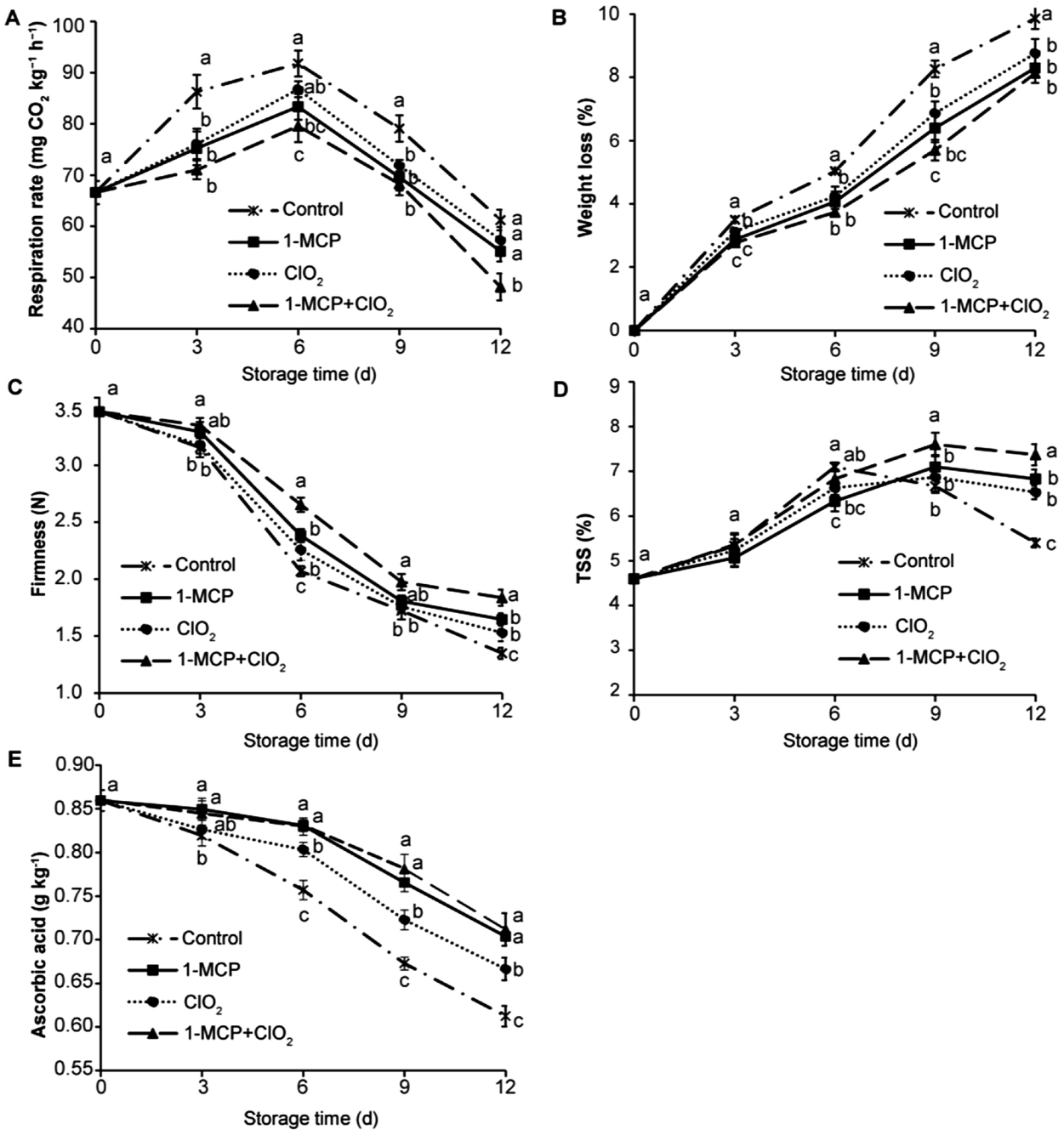


**Fig. 1.** Changes in respiration rate (A), weight loss (B), firmness (C), total soluble solid (TSS, D) and ascorbic acid (E) of green pepper fruit after treatment with 1 μL $L^{-1}$ 1-MCP, 30 μL $L^{-1}$ $ClO_2$ and 1 μL $L^{-1}$ 1-MCP+30 μL $L^{-1}$ $ClO_2$ during storage at 20 °C for 12 d. Data are shown as means ± SD of three replicates (15 peppers of each replicate). Different letters means statistically significant difference in the same day during storage ($P < 0.05$).

All above results showed that all treatments could delay the color change and chlorophyll degradation of green pepper during storage. Among all the treatments, 1-MCP + $ClO_2$ was the most effective treatment, and 1-MCP had higher efficacy than $ClO_2$.

### 3.3. *Effect of different treatments on the expression of chlorophyll degradation associated genes in the peel of green pepper*

In order to further investigate the effect of different treatments on chlorophyll degradation in green pepper fruit, the expression levels of *CaCLH*, *CaPPH*, *CaPAO* and Ca*RCCR* genes were analyzed by qRT-PCR. The expression level of the control group on day 0 was defined as 1. Gene expression levels were compared to the control group on day 0 by the $2^{-\Delta\Delta Ct}$ relative quantification method (Livak and Schmittgen, 2001). Fig. 4A showed that *CaCLH* gene expression was not affected by $ClO_2$ treatment. The relative expression of *CaCLH* increased during storage, and there was no significant difference between the control and $ClO_2$ treated group during the storage period ($P > 0.05$). However, the mRNA levels of *CaCLH* were substantially down-regulated by 1-MCP and 1-MCP + $ClO_2$. The relative expression levels of *CaPPH* and *CaPAO* are depicted in Fig. 4B and C. During storage, the expression of *CaPPH* and *CaPAO* in the control group increased rapidly and then gradually decreased, whereas 1-MCP, $ClO_2$ and 1-MCP + $ClO_2$ treatment groups showed an increasing trend. Significant differences were observed among different groups ($P < 0.05$). This indicated that all treatments could delay the peak time and suppress the expressions of *CaPPH* and

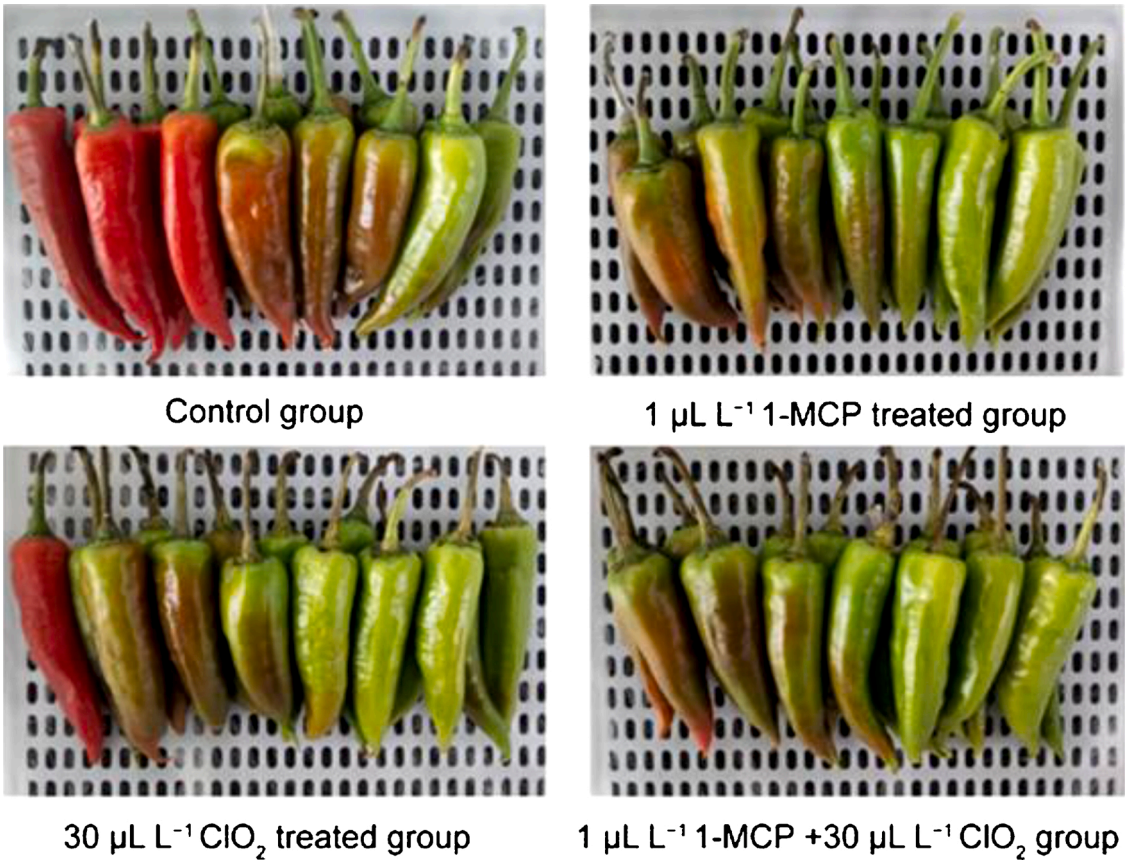


**Fig. 2.** Color phenotype of green pepper fruit in the control group and 1-MCP, $ClO_2$, 1-MCP + $ClO_2$ treatment after storage at 20 °C for 12 d. Each group contained 15 peppers and experiment was performed in triplicate.

*CaPAO*. As shown in Fig. 4D, the highest expression of *CaRCCR* in the control group appeared on day 3, while the peak time of all treatment groups was postponed to day 9. Moreover, all treatments markedly inhibited the expression of *CaRCCR*. All results suggested that1-MCP, $ClO_2$ and 1-MCP + $ClO_2$ treatments could retard chlorophyll degradation by suppressing the expression of genes involved in the chlorophyll breakdown pathway. Among all treatments, 1-MCP + $ClO_2$ had the most effective effect, followed by 1-MCP and $ClO_2$.

## 4. Discussion

Changes in the postharvest quality of green pepper determine customer acceptance, so the quality characteristics including respiration rate, weight loss, firmness, TSS, and ascorbic acid were measured in the present work. Previous reports have revealed that 1-MCP has significant effects on delaying ripening processes as shown by decreasing the respiration rate, reducing weight loss, maintaining firmness, TSS and ascorbic acid content (Cao et al., 2012; Ilić et al., 2012). Du et al. (Du et al., 2007) also reported low dose $ClO_2$ could effectively delay the senescence and maintain the quality attributes of green bell peppers during storage. Zhang et al. (2018) found that compared with 1-MCP alone, the combination of 1-MCP and $ClO_2$ was superior in delaying the physiological transformation and improving shelf life quality of green pepper. These are in agreement with the experimental results in this study (Fig. 1). In addition, 1-MCP + $ClO_2$ was the most effective treatment to maintain the postharvest quality of green pepper, followed by 1-MCP and $ClO_2$. Interestingly, a clear climacteric respiration pattern was observed in this study (Fig. 1A), although pepper fruit is generally classified as non-climacteric fruit (Aizat et al., 2013). This may be due to different cultivars (Vijay et al., 2012). There have been reported that sweet pepper (Villavicencio et al., 1999) and "Cesari yellow" hot pepper (Barrera et al., 2008) show climacteric behaviors.

The change in color represents the ripening process of the pepper fruit. With a sharp decrease in chlorophyll content and a simultaneous increase in carotenoid biosynthesis, the color changed from green to red. Current data have shown that the application of 1-MCP alone or in combined with $ClO_2$ can delay the degradation of chlorophyll and maintain the green peel color of fruits and vegetables, such as apple (Lv et al., 2020), pear (Cheng et al., 2012), broccoli (Gong and Mattheis, 2003), avocado (Hershkovitz et al., 2005) and pepper (Zhang et al., 2018). Studies on pears showed that 1-MCP treatment controlled chlorophyll degradation in 'Bosc' (Xie et al., 2017), 'Yali' (Cheng and Guan, 2014), 'Emerald', 'Jingbai' (Cheng et al., 2012) and 'Comice' (Zhao et al., 2020) by inhibiting ethylene action and suppressing the expression of chlorophyll degradation genes. Lv et al. reported that apples treated with 1-MCP presented lower quantities of pheophorbide a oxygenase (PAO), pheophytinase (PPH) and red chlorophyll catabolite reductase (RCCR). Meanwhile, 1-MCP differentially regulated the expression of chlorophyll catabolic genes in apple peel tissues (Lv et al., 2020). In avocado and broccoli, postharvest 1-MCP reduced activity of peroxidase (POX) and inhibited selectively some of the genes encoding enzymes related chlorophyll catabolism (Hershkovitz et al., 2005; Gómez-Lobato et al., 2012; Gong and Mattheis, 2003). Although the effects of 1-MCP on chlorophyll degradation were different among species, in general, the application of 1-MCP can delay chlorophyll degradation by inhibiting the ethylene production rate, reducing peroxidase (POX) and chlorophyllase activity, suppressing the expression of chlorophyll catabolic genes (*CCGs*). $ClO_2$ alone also has stay-green function. It maintained the chlorophyll content in Hangzhou cabbage (Zhen et al., 2017), Shawo green turnips (Li et al., 2015) and green pepper (Du et al., 2007; Wei et al., 2019). Zhang et al. found that the slow release of $ClO_2$ gas maintained the color of the mangos by

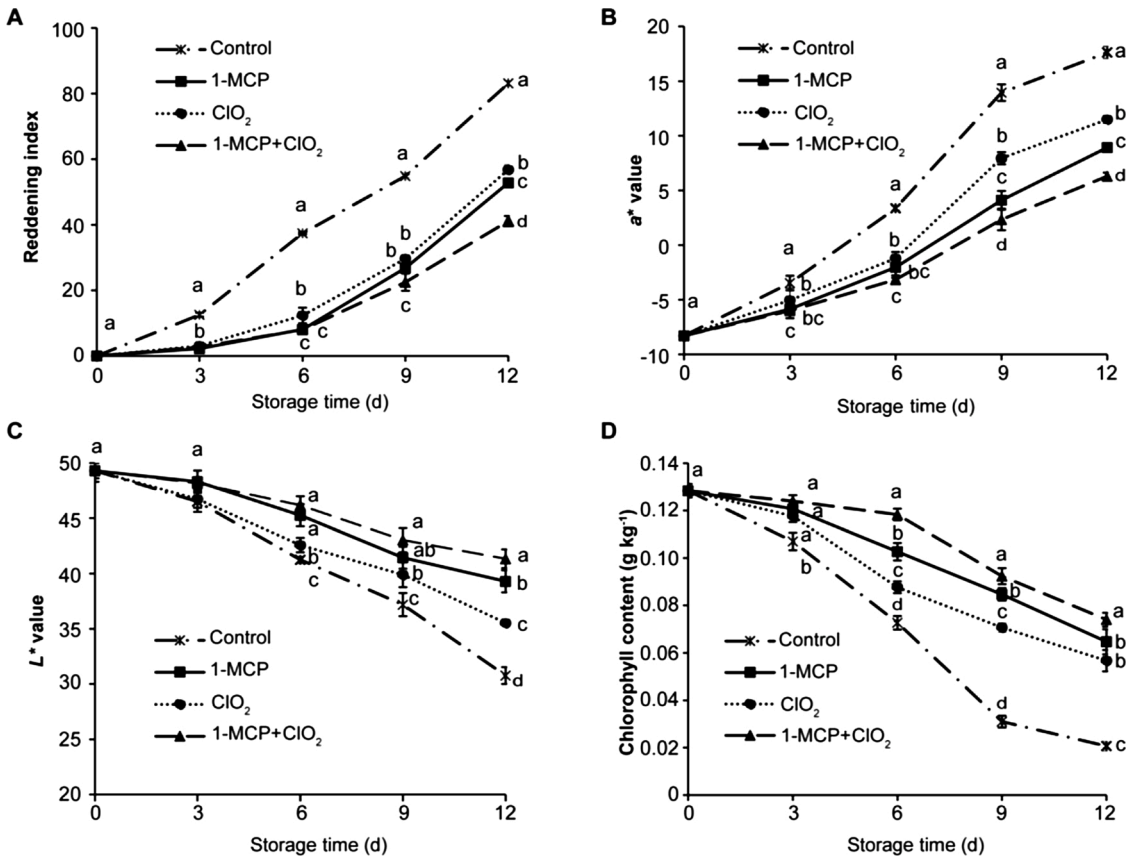


**Fig. 3.** Changes in reddening index (A), $a^*$ value (B), $L^*$ value (C), and chlorophyll content (D) of green pepper fruit after treatment with 1 μL $L^{-1}$ 1-MCP, 30 μL $L^{-1}$ $ClO_2$ and 1 μL $L^{-1}$ 1-MCP+30 μL $L^{-1}$ $ClO_2$. Data are shown as means ± SD of three replicates (15 peppers of each replicate). Different letters means statistically significant difference in the same day during storage ($P < 0.05$).

reducing enzyme activity and protecting the chlorophyll from hydrolysis and oxidation. In addition, the application of $ClO_2$ resulted in reduced ethylene production rate and suppressed expression of genes associated with the chlorophyll breakdown in long pepper fruit (Wei et al., 2019). However, as a strong oxidizing agent, high concentrations of $ClO_2$ may cause chlorophyll oxidation, leading to bleaching phenomenon (Gómez-López et al., 2009). Therefore, treatment condition should be optimized to avoid bleaching effects. In this study. 1-MCP, $ClO_2$ and 1-MCP + $ClO_2$ treatments remarkably delay color development and inhibit the decrease of chlorophyll content (Figs. 2 and 3). Significant differences were observed among different treatments ($P < 0.05$). This suggested that the combination of 1-MCP and $ClO_2$ was more effective in maintaining the green color of pepper fruit than 1-MCP or $ClO_2$.

To explore the molecular mechanism of chlorophyll degradation in the green pepper fruit, the expression patterns of *CaCLH*, *CaPPH*, *CaPAO* and *CaRCCR* in response to different treatments were analyzed during postharvest storage (Fig. 4). *CLH* is the first regulation gene. However, its involvement in chlorophyll degradation is still a matter of debate. Recent studies have shown that two *CLHs* present in *Arabidopsis* are dispensable for chlorophyll breakdown during leaf senescence (Schenk et al., 2007; Zhou et al., 2008). In addition, *CLH* had marginal effects on chlorophyll degradation in pear fruit (Cheng and Guan, 2014). By contrast, several reports in different species support the correlation between *CLH* and chlorophyll degradation. Transgenic broccoli (*Brassica oleracea* var *italica*) with antisense BoCLH1 delayed the rate of postharvest chlorophyll degradation (Chen et al., 2008). Furthermore, *citrus CLH* was shown to be involved during ethylene-induced ripening and overexpression of citrus *CLH* in squash (*Cucurbita pepo*) and tobacco (*Nicotiana tabacum*) led to enhanced chlorophyll breakdown (Harpaz--Saad et al., 2007; Shemer et al., 2008). Therefore, the role of *CLH* in the chlorophyll degradation pathway may vary from species to species. In this study, *CaCLH* expression was up-regulated during storage and it was positively correlated with chlorophyll degradation. In all treatments, *CaCLH* gene expression was not affected by $ClO_2$ treatment, which is consistent with the report of Wei et al. (Wei et al., 2019). However, the expression of *CaCLH* was significant suppressed by 1-MCP and 1-MCP + $ClO_2$. Thus, it indicated that 1-MCP and $ClO_2$ played different regulatory roles in the chlorophyll degradation pathway. Previous reports have demonstrated that other three enzymes, PPH, PAO and RCCR are also involved in the chlorophyll catabolic pathway. PPH is required for chlorophyll dephytilation and the absent of *PPH* causes a stay-green phenotype during leaf senescence (Schelbert et al., 2009). While PPH also participated in chlorophyll degradation in fruits and vegetables. In our work, the expression patterns of *CaPPH* in all treatments closely related to changes in chlorophyll content (Figs. 3D and 4 B). These results are in consistent with findings reported for pear (Comice), apple (*Malus* × *domestica Borkh.*) and broccoli (*Brassica oleracea* L.), suggesting that *PPH* may be a key player in senescence-induced chlorophyll degradation (Gómez-Lobato et al., 2012; Lv et al., 2020; Zhao et al., 2020). PAO and RCCR were found to be active in the chromoplast membranes of tomato and bell pepper fruits (Akhtar et al., 1999; Moser and Matile, 1997), indicating their important role in fruit ripening. According to Wei et al. (Wei et al., 2019), the expressions of *CaPAO* and *CaRCCR* in green pepper were increased rapidly and then gradually decreased during storage. Our data showed the similar expression pattern of *CaPAO* and *CaRCCR*. Moreover, 1-MCP, $ClO_2$ and 1-MCP + $ClO_2$ treatment markedly reduced the expression levels of *CaPPH*, *CaPAO* and *CaRCCR* and postponed the peak time. Except for *CaCLH*, all aforementioned gene expression were suppressed by 1-MCP, $ClO_2$ and 1-MCP + $ClO_2$ treatments, and all treatments delayed the chlorophyll

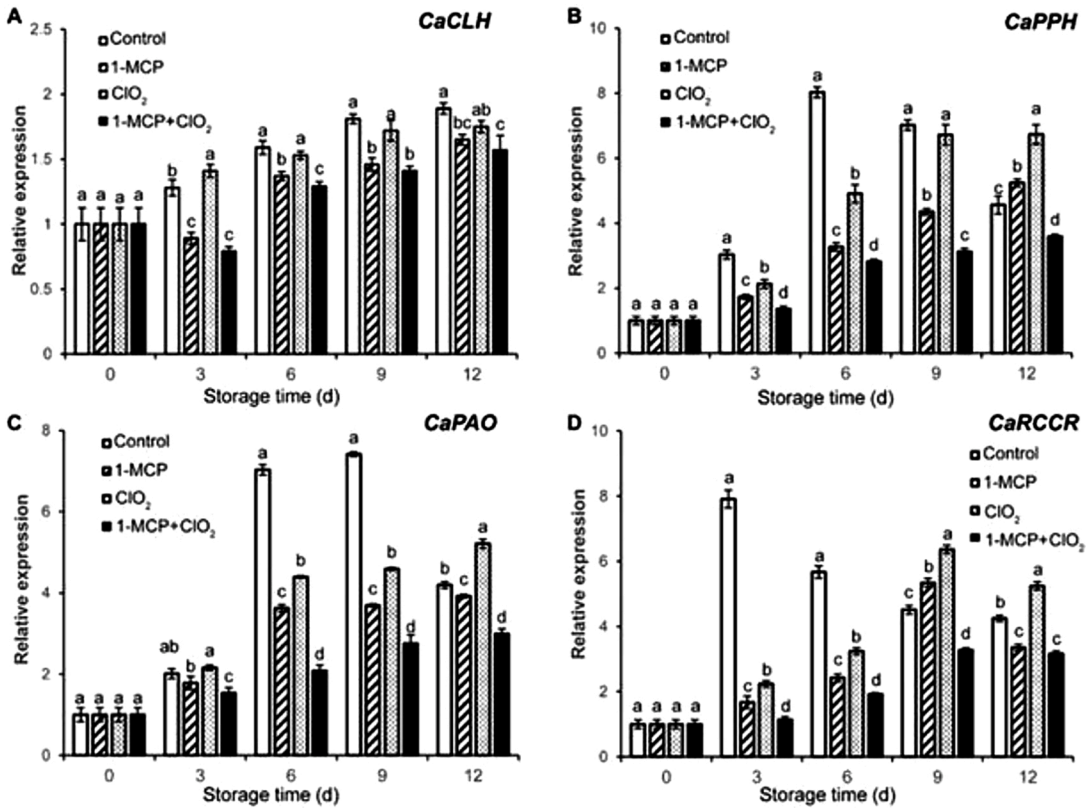


**Fig. 4.** Effect of 1 μL $L^{-1}$ 1-MCP, 30 μL $L^{-1}$ $ClO_2$ and 1 μL $L^{-1}$ 1-MCP+30 μL $L^{-1}$ $ClO_2$ treatment on the expression of chlorophyll degradation associated genes in green pepper fruit (A: *CaCLH*; B: *CaPPH*; C: *CaPAO*; D:*CaRCCR*). Expression value of chlorophyll degradation associated genes in 0 d storage was set to 1. Data are shown as means ± SD of three replicates (15 peppers of each replicate). Different letters means statistically significant difference in the same day during storage ($P < 0.05$).

breakdown and color changes during storage, thus we speculate that 1-MCP, $ClO_2$ and 1-MCP + $ClO_2$ treatment may delay chlorophyll degradation by suppressing the expression of genes involved in the chlorophyll breakdown pathway. For *CaCLH*, 1-MCP and $ClO_2$ exhibit different inhibitory effects, indicating their different regulation roles on the chlorophyll degradation pathway. The effects of different treatments on suppressing gene expression are ranked as follows: 1-MCP + $ClO_2$ > 1-MCP > $ClO_2$.

## 5. Conclusion

Postharvest application of 1-MCP, $ClO_2$ and 1-MCP + $ClO_2$ have significant effects on inhibiting color changes, decreasing the respiration rate, reducing weight loss, maintaining firmness, TSS and ascorbic acid content. Additionally, all treatments can effectively delay the chlorophyll degradation by suppressing the expression of genes involved in the chlorophyll breakdown pathway. Interestingly, 1-MCP and $ClO_2$ play different regulation roles on the chlorophyll degradation pathway as they have different inhibitory effects on *CaCLH* expression. Moreover, the data obtained in this study suggested that compared with 1-MCP and $ClO_2$ alone, 1-MCP + $ClO_2$ was superior in maintaining postharvest quality and inhibiting the expression of chlorophyll degradation associated genes during green pepper storage. Therefore, 1-MCP + $ClO_2$ treatment may serve as an efficient method to preserve other perishable products.

## CRediT authorship contribution statement

**Yamin Du:** Conceptualization, Investigation, Writing - original draft. **Tong Jin:** Investigation. **Handong Zhao:** Investigation, Writing - review & editing. **Cong Han:** Investigation. **Fei Sun:** Data curation. **Qingmin Chen:** Conceptualization, Methodology, Supervision. **Fengli Yue:** Methodology. **Zisheng Luo:** Writing - review & editing. **Maorun Fu:** Conceptualization, Writing - review & editing, Supervision.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

This work was supported by the Outstanding Youth Science Foundation in Shandong Province(Project No. ZR2019YQ16), the Youth Science and Technology Innovation Team of Shandong Province (Project No. 2019KJF01), the Agricultural Industrial Technology System of Shandong Province (Project No. SDAIT-05-14) and the Natural Science Foundation of Shandong Province (ZR2018PC032) in China.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.postharvbio.2020.111363.

## References

Aizat, W.M., Able, J.A., Stangoulis, J.C.R., Able, A.J., 2013. Characterisation of ethylene pathway components in non-climacteric capsicum. BMC Plant Biol. 13, 191. https://doi.org/10.1186/1471-2229-13-191.

Akhtar, M.S., Goldschmidt, E.E., John, I., Rodoni, S., Matile, P., Grierson, D., 1999. Altered patterns of senescence and ripening in gf, stay green mutant of tomato

(*Lycopersicon esculentum Mill.*). J. Exp. Bot. 50, 1115–1122. https://doi.org/10.1093/jexbot/50.336.1115.
Barrera, J.A., Hernandez, M.S., Carrillo, M., Obando-Ulloa, J.M., Fernandez-Trujillo, J.P., Martinez, O., 2008. Modeling fruit growth and ripening and quality traits of climacteric and non-climacteric amazonic hot pepper accessions. Acta Hortic. 802, 325–330. https://doi.org/10.17660/actahortic.2008.802.42.
Bower, J.H., Biasi, W.V., Mitcham, E.J., 2003. Effects of ethylene and 1-MCP on the quality and storage life of strawberries. Postharvest Biol. Technol. 28, 417–423. https://doi.org/10.1016/S0925-5214(02)00208-9.
Cao, S.F., Yang, Z.F., Zheng, Y.H., 2012. Effect of 1-methylcyclopene on senescence and quality maintenance of green bell pepper fruit during storage at 20°C. Postharvest Biol. Technol. 70, 1–6. https://doi.org/10.1016/j.postharvbio.2012.03.005.
Chen, L.F.O., Lin, C.H., Kelkar, S.M., Chang, Y.M., Shaw, J.F., 2008. Transgenic broccoli (*Brassica oleracea var. italica*) with antisense chlorophyllase (*BoCLH1*) delays postharvest yellowing. Plant Sci. 174, 25–31. https://doi.org/10.1016/j.plantsci.2007.09.006.
Cheng, Y., Guan, J., 2014. Involvement of pheophytinase in ethylene-mediated chlorophyll degradation in the peel of harvested' Yali' pear. J. Plant Growth Regul. 33, 364–372. https://doi.org/10.1007/s00344-013-9383-z.
Cheng, Y.D., Dong, Y., Yan, H.B., Ge, W.Y., Shen, C.G., Guan, J.F., Liu, L.Q., Zhang, Y.Y., 2012. Effects of 1-MCP on chlorophyll degradation pathway-associated genes expression and chloroplast ultrastructure during the peel yellowing of Chinese pear fruits in storage. Food Chem. 135, 415–422. https://doi.org/10.1016/j.foodchem.2012.05.017.
Christ, B., Hörtensteiner, S., 2014. Mechanism and significance of chlorophyll breakdown. J. Plant Growth Regul. 33, 4–20. https://doi.org/10.1007/s00344-013-9392-y.
Du, J.H., Fu, M.R., Li, M.M., Xia, W., 2007. Effects of chlorine dioxide gas on postharvest physiology and storage quality of green bell pepper (*Capsicum frutescens L. var.* Longrum). Agric. Sci. China 6, 214–219. https://doi.org/10.1016/S1671-2927(07)60037-6.
Estiarte, N., Crespo-Sempere, A., Marín, S., Sanchis, V., Ramos, A.J., 2016. Effect of 1-methylcyclopropene on the development of black mold disease and its potential effect on alternariol and alternariol monomethyl ether biosynthesis on tomatoes infected with *Alternaria alternata*. Int. J. Food Microbiol. 236, 74–82. https://doi.org/10.1016/j.ijfoodmicro.2016.07.001.
Fernández-Trujillo, J.P., Serrano, J.M., Martínez, J.A., 2009. Quality of red sweet pepper fruit treated with 1-MCP during a simulated post-harvest handling chain. Food Sci. Technol. Int. 15, 23–30. https://doi.org/10.1177/1082013208100464.
Finger, F., Pereira, G., 2016. Physiology and Postharvest of Pepper Fruits, Production and Breeding of Chilli Peppers (*Capsicum* Spp.). Springer, cham, pp. 27–40. https://doi.org/10.1007/978-3-319-06532-8.
Gómez-Lobato, M.E., Hasperué, J.H., Civello, P.M., Chaves, A.R., Martínez, G.A., 2012. Effect of 1-MCP on the expression of chlorophyll degrading genes during senescence of broccoli (*Brassica oleracea L.*). Sci. Hortic. 144, 208–211. https://doi.org/10.1016/j.scienta.2012.07.017.
Gómez-López, V.M., Rajkovic, A., Ragaert, P., Smigic, N., Devlieghere, F., 2009. Chlorine dioxide for minimally processed produce preservation: a review. Trends Food Sci. Technol. 20, 17–26. https://doi.org/10.1016/j.tifs.2008.09.005.
Gong, Y.P., Mattheis, J.P., 2003. Effect of ethylene and 1-methylcyclopropene on chlorophyll catabolism of broccoli florets. Plant Growth Regul. 40, 33–38. https://doi.org/10.1023/A:1023058003002.
Harpaz-Saad, S., Azoulay, T., Arazi, T., Ben-Yaakov, E., Mett, A., Shiboleth, Y.M., Hörtensteiner, S., Gidoni, D., Gal-On, A., Goldschmidt, E.E., Eyal, Y., 2007. Chlorohyllase is a rate-limiting enzyme in chlorophyll catabolism and is posttranslationally regulated. Plant Cell 19, 1007–1022. https://doi.org/10.1105/tpc.107.050633.
Hauenstein, M., Christ, B., Das, A., Aubry, S., Hörtensteiner, S., 2016. A role for TIC55 as a hydroxylase of phyllobilins, the products of chlorophyll breakdown during plant senescence. Plant Cell 28, 2510–2527. https://doi.org/10.1105/tpc.16.00630.
Hershkovitz, V., Saguy, S., Pesis, E., 2005. Postharvest application of 1-MCP to improve the quality of various avocado cultivars. Postharvest Biol. Technol. 37, 252–264. https://doi.org/10.1016/j.postharvbio.2005.05.003.
Ilić, Z., Aharon, Z., Perzelan, Y., Alkalai-Tuvia, S., Fallik, E., 2008. The influence of 1-MCP on chlorophyll, antioxidants activity and quality changes in" Ever-Green" and Red Pepper fruits after harvest. Acta Hortic. 830, 643–650. https://doi.org/10.17660/ActaHortic.2009.830.93.
Ilić, Z., Trajković, R., Perzelan, Y., Alkalai-Tuvia, S., Fallik, E., 2012. Influence of 1-methylcyclopropene (1-MCP) on postharvest storage quality in green bell pepper fruit. Food Bioproc. Tech. 5, 2758–2767. https://doi.org/10.1007/s11947-011-0614-7.
Itai, A., Igori, T., Fujita, N., Egusa, M., Murayama, H., 2012. Ethylene analog and 1-Methylcyclopropene enhance black spot disease development in Pyrus pyrifolia nakai. Hortsci. A Publ. Am. Soc. Hortic. Sci. 47, 228–231. https://doi.org/10.21273/HORTSCI.47.2.228.
Janisiewicz, W.J., Leverentz, B., Conway, W.S., Saftner, R.A., Camp, M.J., 2003. Control of bitter rot and blue mold of apples by integrating heat and antagonist treatments on 1-MCP treated fruit stored under controlled atmosphere conditions. Postharvest Biol. Technol. 29, 129–143. https://doi.org/10.1016/S0925-5214(03)00040-1.
Jibran, R., Sullivan, K.L., Crowhurst, R., Erridge, Z.A., Chagné, D., McLachlan, A.R.G., Brummell, D.A., Dijkwel, P.P., Hunter, D.A., 2015. Staying green postharvest: how three mutations in the Arabidopsis chlorophyll *b* reductase gene *NYC1* delay degreening by distinct mechanisms. J. Exp. Bot. 66, 6849–6862. https://doi.org/10.1093/jxb/erv390.
Li, Z.Q., Zhao, Y.L., Hu, H.Y., Cui, H.Y., Li, P., 2015. The effects of different concentrations of chlorine dioxide for fresh-cut Shawo green turnip storage quality. Farm Products Processing 390, 13–16. https://doi.org/10.3969/j.issn.1671-9646(X).2015.08.032.
Li, Z., Wu, S., Chen, J., Wang, X., Gao, J., Ren, G., Kuai, B., 2017. NYEs/SGRs-mediated chlorophyll degradation is critical for detoxification during seed maturation in *Arabidopsis*. Plant J. 92, 650–661. https://doi.org/10.1111/tpj.13710.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262.
Lv, J.Y., Zhang, M.Y., Bai, L., Han, X.Z., Ge, Y.H., Wang, W.H., Li, J.R., 2020. Effects of 1-methylcyclopropene (1-MCP) on the expression of genes involved in the chlorophyll degradation pathway of apple fruit during storage. Food Chem. 308, 125707 https://doi.org/10.1016/j.foodchem.2019.125707.
Maalekuu, K., Elkind, Y., Tuvia-Alkalai, S., Shalom, Y., Fallik, E., 2004. The influence of harvest season and cultivar type on several quality traits and quality stability of three commercial sweet bell peppers during the harvest period. Adv. Hortic. Sci. 21–25. https://doi.org/10.2307/42882283.
Matile, P., Hörtensteiner, S., Thomas, H., 1999. Chlorophyll degradation. Annu. Rev. Plant Biol. 50, 67–95. https://doi.org/10.1146/annurev.arplant.50.1.67.
Meguro, M., Ito, H., Takabayashi, A., Tanaka, R., Tanaka, A., 2011. Identification of the 7-hydroxymethyl chlorophyll *a* reductase of the chlorophyll cycle in *Arabidopsis*. Plant Cell 23, 3442–3453. https://doi.org/10.1105/tpc.111.089714.
Moser, D., Matile, P., 1997. Chlorophyll breakdown in ripening fruits of *Capsicum annuum*. J. Plant Physiol. 150, 759–761. https://doi.org/10.1016/S0176-1617(97)80296-9.
Porat, R., Weiss, B., Cohen, L., Daus, A., Goren, R., Droby, S., 1999. Effects of ethylene and 1-methylcyclopropene on the postharvest qualities of 'Shamouti'oranges. Postharvest Biol. Technol. 15, 155–163. https://doi.org/10.1016/S0925-5214(98)00079-9.
Pružinská, A., Anders, I., Aubry, S., Schenk, N., Tapernoux-Lüthi, E., Müller, T., Kräutler, B., Hörtensteiner, S., 2007. In vivo participation of red chlorophyll catabolite reductase in chlorophyll breakdown. Plant Cell 19, 369–387. https://doi.org/10.1105/tpc.106.044404.
Rodoni, L., Vicente, A., Azevedo, S., Concellón, A., Cunha, L.M., 2015. Quality retention of fresh-cut pepper as affected by atmosphere gas composition and ripening stage. LWT-Food Sci. Technol. 60, 109–114. https://doi.org/10.1016/j.lwt.2014.08.023.
Roongruangsri, W., Rattanapanone, N., Leksawasdi, N., Boonyakiat, D., 2013. Influence of storage conditions on physico-chemical and biochemical of two tangerine cultivars. J. Agric. Sci. 5, 70. https://doi.org/10.5539/jas.v5n2p70.
Rüdiger, W., 2002. Biosynthesis of chlorophyll *b* and the chlorophyll cycle. Photosyn. Res. 74, 187–193. https://doi.org/10.1023/A:1020959610952.
Schelbert, S., Aubry, S., Burla, B., Agne, B., Kessler, F., Krupinska, K., Hörtensteiner, S., 2009. Pheophytin pheophorbide hydrolase (pheophytinase) is involved in chlorophyll breakdown during leaf senescence in *Arabidopsis*. Plant Cell 21, 767–785. https://doi.org/10.1105/tpc.108.064089.
Schenk, N., Schelbert, S., Kanwischer, M., Goldschmidt, E.E., Dörmann, P., Hörtensteiner, S., 2007. The chlorophyllases AtCLH1 and AtCLH2 are not essential for senescence-related chlorophyll breakdown in *Arabidopsis thaliana*. FEBS Lett. 581, 5517–5525. https://doi.org/10.1016/j.febslet.2007.10.060.
Shemer, T.A., Harpaz-Saad, S., Belausov, E., Lovat, N., Krokhin, O., Spicer, V., Standing, K.G., Goldschmidt, E.E., Eyal, Y., 2008. Citrus chlorophyllase dynamics at ethylene-induced fruit color-break: a study of chlorophyllase expression, posttranslational processing kinetics, and in Situ intracellular localization. Plant Physiol. 148, 108–118. https://doi.org/10.1104/pp.108.124933.
Vijay, P., Rakesh, P., Srivastava, G., 2012. The fading distinctions between classical patterns of ripening in climacteric and non-climacteric fruit and the ubiquity of ethylene—an overview. J. Food Sci. Technol. 49, 1–21. https://doi.org/10.1007/s13197-011-0293-4.
Villavicencio, L., Blankenship, S.M., Sanders, D.C., Swallow, W.H., 1999. Ethylene and carbon dioxide production in detached fruit of selected pepper cultivars. J. Am. Soc. Hortic. Sci. 124, 402–406. https://doi.org/10.21273/JASHS.124.4.402.
Wang, Q., Ding, T., Zuo, J.H., Gao, L.P., Fan, L.L., 2016. Amelioration of postharvest chilling injury in sweet pepper by glycine betaine. Postharvest Biol. Technol. 112, 114–120. https://doi.org/10.1016/j.postharvbio.2015.07.008.
Wei, F., Fu, M.R., Li, J.P., Yang, X.Y., Chen, Q.M., Tian, S.P., 2019. Chlorine dioxide delays the reddening of postharvest green peppers by affecting the chlorophyll degradation and carotenoid synthesis pathways. Postharvest Biol. Technol. 156, 110939 https://doi.org/10.1016/j.postharvbio.2019.110939.
Xie, X.B., Fang, C.B., Wang, Y., 2017. Inhibition of ethylene biosynthesis and perception by 1-methylcyclopropene and its consequences on chlorophyll catabolism and storage quality of 'Bosc' pears. J. Am. Soc. Hortic. Sci. 142, 92–100. https://doi.org/10.21273/JASHS04017-16.
Yan, R., Chen, Q.M., Fu, M.R., 2019. Chlorine dioxide generation method and its action mechanism for removing harmful substances and maintaining quality attributes of agricultural products. Food Bioproc. Tech. 12, 1110–1122. https://doi.org/10.1007/s11947-019-02279-x.
Yang, X.Y., Zhang, X.H., Fu, M.R., Chen, Q.M., Muzammil, J.M., 2018. Chlorine dioxide fumigation generated by a solid releasing agent enhanced the efficiency of 1-MCP treatment on the storage quality of strawberry. J. Food Sci. Technol. 55, 2003–2010. https://doi.org/10.1007/s13197-018-3114-1.
Zhang, X.M., Fu, M.R., 2018. Inhibitory effect of chlorine dioxide ($ClO_2$) fumigation on growth and patulin production and its mechanism in Penicillum expansum. LWT-Food Sci. Technol. 96, 335–343. https://doi.org/10.1016/j.lwt.2018.05.051.
Zhang, X.M., Li, J.P., Fu, M.R., Jin, T., Yang, X.Y., Han, C., Chen, Q.M., Yue, F.L., 2018. Effects of the combination of 1-MCP and $ClO_2$ on shelf life quality of green pepper. Sci. Technol. Food Ind. 39, 275–280. https://doi.org/10.13386/j.issn1002-0306.2018.13.050.

Zhang, B.D., Huang, C.X., Zhang, L.Y., Wang, J., Huang, X.Q., Zhao, Y., Liu, Y., Li, C.C., 2019. Application of chlorine dioxide microcapsule sustained-release antibacterial films for preservation of mangos. J. Food Sci. Technol. 56, 1095–1103. https://doi.org/10.1007/s13197-019-03636-6.

Zhao, J., Xie, X., Wang, S., Zhu, H., Fang, C., 2020. 1-Methylcyclopropene affects ethylene synthesis and chlorophyll degradation during cold storage of' Comice' pears. Sci. Hortic. 260, 108865 https://doi.org/10.1016/j.scienta.2019.108865.

Zhen, F.Y., Qiao, Y.J., Gao, C.X., Wang, X., 2017. Effect of chlorine dioxide gas on preservation of Hangzhou cabbage. J. Nucl. Agric. Sci. 31, 1323–1329. https://doi.org/10.11869/j.issn.100-8551.2017.07.1323.

Zhou, X., Liao, Y., Ren, G.D., Zhang, Y.Y., Kuai, B.K., 2008. Repression of AtCLH1 expression results in a decrease in the ratio of chlorophyll *a*/*b* but doesn't affect the rate of chlorophyll degradation during leaf senescence. J. Plant Physiol. Mol. Biol. 33, 596–606. PMID: 18349515.