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Effects of Nano TiO_2 Cold Plasma Treatment on the Storage Quality of Postharvest Tomatoes

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Abstract

Tomatoes are widely consumed worldwide due to their attractive color, unique flavor, and high levels of antioxidants such as lycopene and carotenoids. However, postharvest tomatoes are highly perishable because of their high moisture content and respiration rate, which accelerate chlorophyll degradation, carotenoid accumulation, softening, and color change during storage. These changes lead to significant economic losses. In this study, the effects of atmospheric cold plasma (ACP) treatment at different intensities (40, 60, and 80 kV) on the storage quality and chlorophyll metabolism of postharvest tomatoes were investigated. Tomatoes were treated with ACP for 5 minutes and stored at 10 ± 0.5 °C for 35 days. Several quality parameters including respiration intensity, firmness, total soluble solids (TSS), titratable acidity (TA), and weight loss rate were evaluated during storage. In addition, the activities of chlorophyll degradation enzymes and the expression of key genes related to chlorophyll metabolism (CLH1, PPH, PAO, and RCCR) were analyzed. The results showed that ACP treatment significantly inhibited respiration intensity and weight loss while maintaining higher firmness, TSS, and TA contents compared with the control group. Furthermore, ACP treatment slowed chlorophyll degradation and carotenoid accumulation, thereby delaying the red ripening process of tomatoes. Among the tested treatments, the 60 kV ACP treatment demonstrated the most effective preservation performance by significantly suppressing chlorophyllase and PAO enzyme activities and downregulating the expression of CLH1, PPH, and RCCR genes. These findings indicate that ACP treatment, particularly at 60 kV, is a promising non-thermal and chemical-free technology for improving the postharvest quality and extending the shelf life of tomatoes.

Keywords : Tomato, cold plasma

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Introduction:

Tomatoes are popular across the world because of their unique flavor, bright color, and manyantioxidants, such as lycopene, carotenoids, and chlorophyll [1,2]. However, the chlorophyll content and physical quality of postharvest tomatoes are decreased due to their high degree ofmoistureandrespiration with the extension of the storage time, which results in the tomatoes turning red and softening, which induces economic losses [3]. Although the traditional chemical preservatives can prolong the storage period of postharvest tomatoes, they have no significant effect in inhibiting tomatoes from turning red, and the residue of chemical preservatives is a serious food safety problem [4]. Atmospheric cold plasma (ACP) is a kind of unstable ionized gas, which contains ac tive substances with bacteriostasis. Additionally, ACP treatment is a potential green preser vation technology because of it is less destructive and has no residues [5]. Min et al. [6] reported that the microbes on the surfaces of tomatoes treated with ACP grew slowly, and the sensory quality was well maintained. Zhou et al. [7] found that the microbial growth rate on the surface of cantaloupe was limited by ACP treatment. Similar results were found in studies of blueberries [8], red currants [9], strawberries [10], apples [11], and mulberry [12].

The degradation of chlorophyll and the accumulation of carotenoids are signs of the process through which tomatoes ripen and turn red. Studies have shown that the degradation of chlorophyll is closely related to the combined regulation of the chlorophyll enzyme, pheophorbide a mono-oxygenase (PAO), and the chlorophyll enzyme gene, PAO gene, chlorophyll hydrolase gene (PPH), and red chlorophyll catabolism reductase gene (RCCR) [13–15]. Zhao et al. [16] found that the slowing of chlorophyll degradation in cherry stems was due to the inhibition of PAO and RCCR gene expression. Additionally, the downregulation of PPH and PAO expression slowed the decrease in chlorophyll in the “Comice” pear [17]. In addition, studies have also shown that ACP treatment can induce the type II decomposition of chlorophyll in kiwifruit, resulting in a decrease in the content of chlorophyll A [18]. Baier et al. [19] found that the chlorophyll fluorescence imaging of cucumber was affected by cold plasma treatment. Our study also found that plasma treatment had a positive effect on the green maintenance of postharvest tomatoes, but the effects of ACP treatment on the chlorophyll metabolism and related genes in tomatoes have hardly been studied. The purpose of this study was to explore the effects of different intensities of ACP plasma treatment on the postharvest tomato storage quality and to clarify the most suitable ACP treatment intensity and the influence of the ACP treatment on

chlorophyll metabolism in postharvest tomato fruits. This study can provide a basis for the application of ACP preservation technology to postharvest tomatoes

2. Materials and Methods

2.1. Tomato Preparation

The green ripening tomato variety used in the trial was Aomei, which was harvested in June 2021 from Baodi, Tianjin, China. Tomatoes in the green fruit stage in shock ab sorption packaging were immediately shipped to the National Agricultural Products Preservation Engineering Technology Research Center (Tianjin, China). The tomatoes were screened and randomly grouped into 12 portions per group, and 20 fruits were used for each measurement.

2.2. Plasma Device and Treatment

The ACPgenerator was produced by the National Agricultural Products Preservation Engineering Technology Research Center (Tianjin, China) and consisted of a high-voltage power supply and a dual-dielectric discharge module. The power system voltage range was 0–100 kV. The working gas used during the test was air. The tomatoes were treated with ACP at 0 kV, 40 kV, 60 kV, and 80 kV for 5 min and then stored in cold storage at 10 ±0.5 ◦C, and the samples were taken in sequence at 0 day, 7 day, 14 day, 21 day, 28 day, and 35 day for observation, with 0 day as the initial value of the observed index

2.3. Firmness

The firmness of the tomatoes was measured using a texture analyzer (TA. XT plus, Stable Micro Systems Ltd., Surrey, Godalming, UK) with a probe diameter of 3 mm and a penetration depth of 1 cm, and 3 points were measured for each fruit, and the results were expressed in kg/cm2. 2.4.

2.4. Chlorophyllase, Pheophorbide a Mono-Oxygenase

The activities of the chlorophyllase and pheophorbide a mono-oxygenase were deter mined using a plant chlorophyllase ELISA kit (Shanghai Jianglai Biotechnology Co., Ltd., Shanghai, China). U indicates the magnitude of the enzyme activity. Under the optimal

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conditions (25 ◦C), the number of enzymes required to catalyze 1 micromole (µmol) of the substrate into product per minute is defined as one unit of activity.

2.5. Expression of the Key Enzyme Genes of Chlorophyll Metabolism

RNAextraction was performed on the tomatoes according to the Trizol method of Wanget al. [24], and reverse transcription was performed with an amount of 1 µg of RNA. Based on the mRNA sequences of the tomato genes (GADPH, PAO, PPH, CLH1, CLH2, CLH3, CLH4, RCCR) in the GeneBank, the internal reference gene was selected as GAPDH, and the primer sequences (5-3) used are shown in Table 1. All the genes were subjected to real-time fluorescence quantitative PCR (CFX96 quantitative PCR instrument, Biole Life Medical Products (Shanghai) Co., Ltd., Shanghai, China), and the relative RNA expression was calculated by the $2^{-\Delta\Delta Ct}$ method. 2.12. Statistical Analysis of the Results The measurements were repeated three times for each treatment. The data obtained from the experiments were expressed as the mean ± standard deviation (SD), using Origin 2017 (Origin Lab, Northampton, MA, USA) for the graphing and statistical analysis and IBM SPSS Statistics (version 22.0, Chicago, IL, USA) for the statistical analysis. ANOVA with Tukey's test was used to analyze the significance of the data with the confidence level of $p \leq 0.05$. Pearson correlations were also analyzed using IBM SPSS Statistics (version 22.0, Chicago, IL, USA) at the level of $p < 0.05$ and $p < 0.01$ for significant and relatively significant correlations, respectively

3. Results and Discussion 3.1. Effects of Different Intensities of the ACP Treatment on the Respiration Intensity, Firmness, TSS Content, TA Content, and Weight Loss Rate of Tomatoes The respiratory intensity of the tomatoes during storage is shown in Figure 1A. The respiratory intensity first increased and then decreased among the control and treatment groups and reached its peak after 14 d of storage. The respiratory intensity of the 60 kV treatment group was significantly lower than that of the 40 kV and 80 kV treatment groups and the control group during the whole storage period ($p < 0.05$), indicating that the respiration of the postharvest tomatoes was inhibited by the 60 kV plasma treatment. Misra et al. [10] also found that the respiratory intensity of postharvest strawberries was reduced in the ACP treatment group. The reason for the higher respiratory intensity in the 80 kV treatment group may be the fact that the 80 kV treatment produced more active substances and created an environment higher in reactive oxygen species compared with the 40 kV and 60 kV treatments. Zhang et al. found that high-intensity ACP treatment produced more active substances [25]. As shown in Figure 1B, the firmness of the tomatoes decreased with

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the prolongation of the storage time, and the firmness of the control group wassignificantly lower than that of the treatment group ($p < 0.05$). In addition, the hardness of the 60 kV treatment group was significantly higher than that of the 40 kV and 80 kV treatment groups, which may be because the 60 kV treatment group had a low respiration intensity and low consumption of pectin and other substances. Zhou et al. [7] also found that the hardness of cantaloupe after cold plasma treatment was maintained at a high level compared with the control group. The total soluble solids (TSS) and titratable acid (TA) content are important indicators for predicting the quality of postharvest tomatoes [26]. As shown in Figure 1C, D, the TSS content of the tomatoes showed an upward trend in the early storage period and a downward trend after 14 d, while the TA content showed a downward trend over the whole storage time. This may be due to the degradation of the macromolecular carbohydrates that are stored in tomatoes into soluble sugars and organic substances with the increase in respiration [27]. The TSS and TA contents of the control group were significantly lower than those of the treatment group, except for the 7 dsample ($p < 0.05$), which suggested that the plasma treatment had a positive effect on the accumulation of TSS and TA in the postharvest tomatoes. Additionally, the TSS and TAcontents were more abundant in the 60 kV treatment group compared to the 40 kV and 80 kVtreatment groups ($p < 0.05$), which may be because the 60 kV treatment suppressed the respiration and maintained lower metabolic levels. On the other hand, the consumption of TSS and TAbypostharvest tomatoes was accelerated at a higher level of reactive oxygen species (ROS) stress [28], which may explain why the TSS and TA contents in the 80 kV treatment group were lower than those in the 60 kV treatment group [29]. Dong et al. [30] also found that the TSS content in blueberries after cold plasma treatment increased by 1.5 times compared with the control group. The decrease in the TA content of kiwifruit was slowed down bycold plasma treatment [18]. Li et al. [31] also discovered that the TA content of fresh-cut strawberries decreased slowly after ACP treatment.

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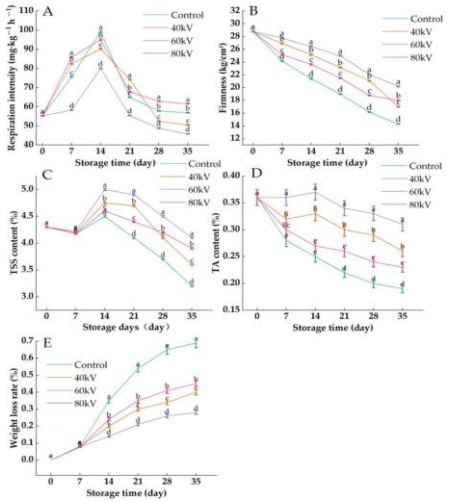

Figure1.EffectsofdifferentintensitiesofACPtreatmentontherespirationintensity,firmness,TSS content,TAcontent,andweightlossrateoftomato: (A)respirationintensity;(B)firmness;(C)TSS content;(D)TAcontent;(E)weightlossrate.Barslabeledwithdifferentsmallletters(a–d)indicate significantdifferencesamongdifferentACPtreatmentsatthesamestoragetime($p \leq 0.05$).

AsshowninFigure1E,theweightlossrateofthepostharvesttomatoesincreased graduallywiththeprolongationofthestoragetime,butitwassimilarbetweenthecontrol groupandtreatmentgroupduringthefirst7day.Thismaybeduetothelowrespiration intensityofthetomatoesandtheslowconsumptionofsubstancesduringtheearlystorage time.Aftertherespiratorypeakappeared,theweightlossrateofthecontrolgroupwas significantly higher than that of the treatment groups

Conclusions

The results showed that the respiration intensity and weight loss of the postharvest tomatoes were inhibited in the ACP treatment groups, and the ACP treatment maintained a high TSS content and TA content compared with the control group. In addition, a higher brightness and lower red transition rates were found in the ACP-treated group. Moreover, the 60 kV ACP treatment significantly inhibited the degradation of chlorophyll, the accumulation of carotenoids, and the activities of chlorophyllase and PAO, and it significantly downregulated the relative expression of CLH1, PPH, and RCCR. Among the three treatment groups, the 60 kV ACP treatment was the appropriate plasma treatment intensity for maintaining high-quality postharvest tomatoes. These results provide some basic data for the future application of ACP to postharvest tomatoes. Additionally, it is

necessary to further analyze the mechanism of the effect of ACP on the storage quality of postharvest tomatoes from a variety of perspectives

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