Optimizing chlorine dioxide treatment for enhanced post-harvest storage quality of *Toona sinensis* (#102095)

First revision

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Optimizing chlorine dioxide treatment for enhanced post-harvest storage quality of *Toona sinensis*

Zeng Rui2 Corresp., 1, 2, 3 , **Yunhong Gao** [4] , **Mingmin Zheng** [2, 3] , **Lai Min** [1, 2] , **Yishi JiGu** [2] , **Jiayu Chen** [2] , **Yun Pei** [2] , **Muhammad Umer Farooq** [5] , **Khalid Mashay Al-Anazi** [6] , **Mohammad Abul Farah** [6]

[1] Institute of Food Fermentation, Chengdu Normal University, Chengdu, 611130, Sichuan, China, chengdu, China

[2] College of Chemistry and Life Science, Chengdu Normal University, Chengdu 611130, Sichuan, China, Chengdu, China

[3] Sichuan Provincial Key Laboratory for Development and Utilization of Characteristic Horticultural Biological Resources, College of Chemistry and Life Sciences, Chengdu Normal University, Chengdu 611130, Sichuan, China, chengdu, China

[4] Sichuan Yizhong Agricultural Development Company, Chengdu 610213, Sichuan, China, chengdu, China

[5] Senior Scientist, Agriculture Department, Ayub Agricultural Research Institute Faisalabad, 38850, Punjab, Pakistan, Faisalabad, Pakistan

[6] Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia, Riyadh, Saudi Arabia

Corresponding Author: Zeng Rui2
Email address: zengrui829@126.com

This study investigated the impact of chlorine dioxide (ClO_2) treatment on the storage quality of *Toona sinensis* after harvesting. *Toona sinensis* samples were treated with different concentrations (0.2, 0.4, 0.8, 1.6 mg/L) of chlorine dioxide and stored at (4±1) ℃, with sampling test every two days. The changes in sensory, nutritional, and chlorine dioxide residues of *Toona sinensis* were estimated regularly. Results revealed that moderate (0.4 ~ 0.8 mg/L) chlorine dioxide concentrations maintained sensory quality, inhibited *Toona sinensis* weight loss, slowed flavonoid and Vitamin C content depletion, reduced nitrite content, and enhanced superoxide dismutase activity. The chlorine dioxide resides in *Toona sinensis* were below the national standards (< 2.0 mg/kg; GB 5009.244-2016). Overall, 0.4-0.8 mg/L chlorine dioxide treatment exhibited optimal effects on *Toona sinensis*, providing a scientific basis for extended storage and preservation of *Toona sinensis*

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Rui Zeng[1, 2,3*], Yunhong Gao[4], Mingmin Zheng[1,2], Min Lai[1,3], Yishi JiGu[1], Jiayu Chen[1], Yun Pei[1], Muhammad Umer Farooq[5], Khalid Mashay Al-Anazi[6], Mohammad Abul Farah[6]

[1] College of Chemistry and Life Science, Chengdu Normal University, Chengdu 611130, Sichuan, China
[2] Sichuan Provincial Key Laboratory for Development and Utilization of Characteristic Horticultural Biological Resources, College of Chemistry and Life Sciences, Chengdu Normal University, Chengdu 611130, Sichuan, China
[3] Institute of Food Fermentation, Chengdu Normal University, Chengdu, 611130, Sichuan, China
[4] Sichuan Yizhong Agricultural Development Company, Chengdu 610213, Sichuan, China
[5] Wheat Research Institute, Agriculture Department, Ayub Agricultural Research Institute Faisalabad, 38850, Punjab, Pakistan
[6] Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

* Correspondence: zengrui829@126.com

Abstract: This study investigated the impact of chlorine dioxide (ClO_2) treatment on the storage quality of *Toona sinensis* after harvesting. *Toona sinensis* samples were treated with different concentrations (0.2, 0.4, 0.8, 1.6 mg/L) of chlorine dioxide and stored at (4±1) ℃, with sampling test every 2nd day. The changes in sensory, nutritional, and chlorine dioxide residues of *Toona sinensis* were estimated regularly. Results revealed that moderate (0.4 ~ 0.8 mg/L) chlorine dioxide concentrations maintained sensory quality, inhibited *Toona sinensis* weight loss, slowed flavonoid and Vitamin C content depletion, reduced nitrite content, and enhanced superoxide dismutase activity. The chlorine dioxide resides in *Toona sinensis* were below the national standards (< 2.0 mg/kg; GB 5009.244-2016). Overall, 0.4 ~ 0.8 mg/L chlorine dioxide treatment exhibited optimal effects on *Toona sinensis*, providing a scientific basis for extended storage and preservation of *Toona sinensis*.

Key words: *Toona sinensis*, chlorine dioxide, preservation, storage, quality, flavonoid, vitamin C.

INTRODUCTION

Toona sinensis (Toon), commonly known as Chinese toon or Chinese mahogany, is indeed an interesting plant to consider for sustainability, it belongs to Meliaceae family (Peng et al. 2019). It has cultural significance in Chinese cuisine and traditional medicine. The plant parts i.e., roots, stems, leaves, and fruits are valued for their unique flavor and nutritional benefits (Wang et al. 2007). The shoots are used as vegetables (Liao et al. 2007). Additionally, different parts of the tree, such as the mature leaves, bark and seeds, have been used in traditional medicine for various purposes (Guanying et al. 2019). The leaves are pinnate, compound, and alternate, consisting of numerous small leaflets, usually buds are marketed in spring. The immature leaves are reddish-purple color, with a unique taste and a special aroma (Jiang et al. 2020; Peng et al. 2019). Toon extracts and chemical components have good antibacterial, anti-inflammatory, analgesic, antioxidant, neuroprotective, anti-cancer, hypoglycemic and other biological activities (Chanyuan et al. 2020). *Toona sinensis* is beloved for its robust aroma and exceptional nutritional content, however, there are challenges in post-harvest preservation, largely due to seasonal variations. The delicacy of fresh Toon leaves make them susceptible to water loss, wilting, leaf drop, and mold during transportation and storage (Wang et al. 2024b; Xu et al. 2022). These issues not only hinder direct sales and further processing but also limit the economic potential of *Toona sinensis*, diminishing its edibility and safety. Addressing these challenges is crucial for unlocking the full value of this esteemed ingredient and ensuring its availability and quality for consumers (Xin et al. 2019).

Chlorine dioxide (ClO_2) is globally recognized as a potent disinfectant, classified as an A1 disinfectant due to its strong oxidizing and bactericidal properties (Mengqiao et al. 2021). Unlike many other sterilization agents, ClO_2 leaves no harmful residues or odors during the sterilization process, ensuring food safety without compromising flavor or sensory quality (Zhang et al. 2023a). It has been successfully employed in preserving various fruits and vegetables, effectively inhibiting the growth of spoilage bacteria without reacting with fatty acids (Wang et al. 2024a; Zhang et al. 2023b). This preservation method maintains food freshness, minimizes quality loss, preserves appearance, and slows the degradation of essential nutrients like soluble solids, titratable acid, vitamin C (Vc), and chlorophyll. Additionally, it enhances the activity of peroxidase (POD) while inhibiting polyphenol oxidase (PPO), further reducing decay in produce (fruits/vegetables) (Chao et al. 2020; Ganhui et al. 2020; Shengzhi et al. 2020; Xingqiang et al. 2020; Yunfei et al. 2020).

Despite the economic potential of *Toona sinensis*, its value is somewhat limited due to concentrated harvesting periods, short sales windows, and post-harvest storage challenges. Current preservation techniques for *Toona sinensis* encompass physical (Yumeng et al. 2023), chemical (Liqiong et al. 2019; Shaohua et al. 2019), biological (Xihang 2022) and combined methods (Shaohua et al. 2021; Yongqing et al. 2014). In this study, we investigated the effects of varying chlorine dioxide concentrations on several physiological parameters during *Toona sinensis* post-harvest storage. Our goal was to identify an effective method to enhance post-harvest quality and prolong shelf life, thereby contributing to the advancement of the *Toona sinensis* industry and meeting consumer demands.

MATERIALS AND METHODS

Material Collection

Toona sinensis was sourced from the Toona base in Guangming Village, Yaodu Town, Qingbaijiang District, Chengdu City, Sichuan Province. These Chinese toons are al clones and cultivated. Fresh Toona leaves of uniform size, free from pests and diseases, and with intact appearance were selected as raw materials. The length of each selected toon shoot was 5-10 cm. They were promptly transported to the laboratory of Chengdu Normal University within one hour of harvest for further analysis.

The following instruments were utilized in study: Ultraviolet-visible spectrophotometer 752G (Shanghai Jingqi Instrument Co., Ltd.), Ultra-low temperature refrigerator DW-25L300 (AUCMA Co., Ltd.), Desktop refrigerated centrifuge 5810R (Eppendorf, Germany), Large-capacity low-speed centrifuge TDL-5A (Jiangsu Jinyi Instrument Technology Co., Ltd.), Ultrasonic cleaner KH3200B (Kunshan Hechuang Ultrasonic Instrument Co., Ltd.), Analytical electronic balance FA1004 (Shanghai Liangping Instrumentation Co., Ltd.).

Preparation of ClO_2 solution

The solid ClO_2 powder was dissolved in distilled water and stored at room temperature away from light for 3 days to allow the solid ClO_2 powder to completely dissolve. It was then stored at 4°C. The concentration of the ClO_2 solution was determined according to the National Standard for Stable Chlorine Dioxide Solution (GB/T 20783-2006)(20783-2006 2006).

Samples Pretreatment

The selected Toon samples were randomly divided into 5 chlorine dioxide (ClO_2) treatment groups (as shown in Table 1), with each group triplicated. Each sample weighing 100g was placed in a perforated ziploc bag made of polyethylene (PE). The inner walls of the ziploc bags were evenly sprayed with a ClO_2 solution using a sprayer. The bags were promptly sealed and stored at 20±2°C for 2, 4, 6, 8 and 10 days. The permissible standard of ClO_2 in food products is followed, which refers to the GB 5009.244-2016 "National Food Safety Standard of the People's Republic of China Determination of Chlorine Dioxide in Food", requires that the surface residue of ClO_2 treated fruits and vegetables should not exceed 2 mg/kg (5009.244-2016 2016).

Table 1: The ClO_2 treatment groups used in the study.

Treatment groups	ClO_2 Concentration mg/L
CK	0
S1	0.2
S2	0.4
S3	0.8
S4	1.6

Determination of Weight Loss Rate

The weight loss rate was estimated using formula.

$$Weight\ Loss\ Rate = \frac{Toon\ quality\ before\ storage - Toon\ quality\ after\ storage}{Toon\ quality\ before\ storage} \quad (1)$$

Determination of Sensory Index

The browning and decaying are important indicators that affect the sensory perception of Chinese toon. The degree of browning may be directly related to the decomposition rate of chlorophyll. Changes in color and smell are the most intuitive manifestations of quality changes during storage. The comprehensive scoring method assesses the appearance, morphology, texture, and distinctive odor of *Toona sinensis* as evaluation criteria. According to the guidelines outlined in Table 2, each criterion is assigned a score of 25 points, resulting in a total score of 100 points. We selected 20 people to evaluate the Chinese toon, ensuring that the evaluation personnel are fixed each time, and take the average of the scoring from the same people. Evaluators calculate the average sensory evaluation score for each group of *Toona sinensis* based on actual observations. If the sensory score falls below 60 points, it indicates that the *Toona sinensis* has lost its commercial value. Sensory evaluation was carried out in a professional sensory evaluation room.

Table 2 Sensory index scoring on freshness evaluation of *Toona sinensis* by Evaluators

Sensory Index	Scoring	Grading Criteria
Appearance	20	a. The color is bright, and the surface has an oily texture (16~20 points).
		b. The color is bright, and the surface has a slight oily texture (11~15 points).
		c. The appearance is average, and the surface has no oily surface texture (6~10 points).
		d. Browning occurs, no commercial value (0~5 points).
Morphology	30	a. Good morphology, smooth leaf edges, no aging phenomenon (26~30 points).
		b. Good morphology, flat leaf edge, no aging phenomenon (20~25 points).
		c. Leaf micro-curl, a small amount of aging phenomenon (11~19 points).
		d. The leaf curl and aging phenomenon is serious (0~10 points).
Texture	25	a. The stems and leaves are straight, and there is no wilting and decay (21~25 points).
		b. The stems and leaves are relatively straight, and there is no wilting and decay (13~20 minutes).
		c. It rarely wilts and decays, and the leaf edge is a small amount of dry brown (6~12 points).
		d. Most of them wilted and rotted, and the tissues were dry brown (0~5 points).
Distinctive Odor	25	a. Rich (21~25 points)
		b. Stronger (13~20 points)
		c. Light (6~12 points)
		d. Light, with a rotten smell (0~5 points)

Determination of Total Flavonoid Content

For the determination of total flavonoid content, the aluminum nitrate method followed by Chun and colleagues was utilized (Chun-Cui T et al. 2024).

1) Weigh 0.50g of crushed *Toona sinensis* into a centrifuge tube, then add 10mL of 50% ethanol. Sonicate the mixture at a temperature of 60°C for 1 hour. Place the centrifuge tube into a centrifuge and spin it at a speed of 5000r/min for 20 minutes. Extract 1mL of supernatant and transfer it to a colorimetric tube with a stopper. Initially, add 0.3mL of 5% Sodium Nitrite ($NaNO_2$) solution and mix thoroughly. Allow it to stand for 6 minutes. Then, add 0.3mL of 10% Aluminum Nitrate ($Al(NO_3)_3$) solution and mix well, let it stand for another 6 minutes. Add 4 mL of 4%

Sodium Hydroxide (NaOH) solution and adjust the volume with 50% ethanol to 10 mL and shake thoroughly. After standing for 15 minutes, measure the absorbance at a wavelength of 510 nm without adding the extract. Each treatment was conducted in triplicate, and absorbance was measured three times per test.

2) To prepare the standard curve, dissolve 10mg of rutin standard sample in 50% ethanol and transfer it to a 50mL volumetric flask. Dilute it to obtain a 0.2mg/mL rutin standard solution. Then, take aliquots of 0.0, 0.5, 1.0, 1.5, 2.0, and 2.5mL from the standard solution and transfer each to a 10mL volumetric flask. Add $NaNO_2$ solution, $Al(NO_3)_3$ solution, and NaOH solution to each flask, and adjust the volume with ethanol as optimized earlier. Shake the solutions thoroughly and allow them to stand for 15 minutes. Use the solution with 0mL of the rutin standard as the reference. Measure the absorbance at 510nm and plot a standard curve.

3) Total flavonoid calculation formula:

$$Flavonoids\ (mg/g) = \frac{m \times V}{M} \quad (2)$$

where: m represents the total flavonoid content in the 1.0mL sample after ultrasonication (mg/g), V denotes the total volume of the extract (mL), and M is the weight of the *Toona sinensis* sample (g).

Determination of Vitamin C Content

For the determination of Vitamin C (Vc) content the national standard (GB5009.86-2016) procedure was followed (5009.86-2016 2016).

1) Calibrating Ascorbic Acid Solution: Accurately draw 1mL of ascorbic acid standard solution into a 50mL volumetric flask. Add 10mL of oxalic acid solution, stir well, and then titrate with 2,6-dichloroindophenol solution until the solution turns pink, ensuring the color remains stable for 15 seconds without fading. Concurrently, another 10mL of oxalic acid solution is used for a blank test.

2) Sample Determination: Accurately weigh 1.0g of crushed *Toona sinensis*. Add a small amount of oxalic acid solution and transfer it to a 50mL volumetric flask. Adjust the volume with oxalic acid solution, shake thoroughly and filter. Add white clay to the filter and repeat the

filtration process. Collect the solution for later use. Pipette 10.0mL of the filtrate into a conical flask. Titrate with the calibrated 2,6-dichloroindophenol solution until a reddish color appears and remains stable for 15 seconds. Note the amount of dye used.

3) Calculation formula:

$$\text{Titer: } T\ (mg/mL) = \frac{C \times V}{V1 - V0} \tag{3}$$

where: C represents the mass concentration of the ascorbic acid standard solution (mg/mL), while V denotes the volume of the ascorbic acid standard solution (mL). V1 represents the volume of 2,6-dichloroindophenol solution consumed during the titration of the ascorbic acid standard solution (mL), and V0 is the volume of 2,6-dichloroindophenol solution consumed during the titration blank (mL).

$$\text{Vitamin C: } Vc\ (mg/100g) = \frac{(Va - Vb) \times T \times A}{m \times 100} \tag{4}$$

where: Va represents the volume of 2,6-dichloroindophenol solution consumed during the titration of the sample (mL), while Vb denotes the volume of 2,6-dichloroindophenol solution consumed for the titration blank (mL). T signifies the titer of the 2,6-dichloroindophenol solution (mg/mL), A is the dilution factor, and m indicates the mass of the sample (g).

Determination of the Nitrite Content

For the determination of the nitrite content, the national standard (GB 5009.33-2016) procedure was followed (5009.33-2016 2016).

1) Sample Determination: Accurately weigh 2.5g of crushed *Toona sinensis*. Add 6.25mL of saturated borax solution, in a 50mL of water at 70°C. Mix thoroughy and heat the mixture in a boiling water bath for 15 minutes. Remove from heat and allow to cool to room temperature. Transfer the above extract quantitatively to a 100mL volumetric flask, adding 2.5mL of potassium ferrocyanide solution dropwise while rotating the flask. Shake well and then add zinc acetate solution. Adjust the volume to the mark with water, shake well, and let it stand for another 30 minutes before filtering. After filtration, aspirate 20.0mL of the filtrate into a 25mL colorimetric tube. Add 1mL of p-aminobenzene sulfur solution and mix well. After standing for 3-5 minutes, add 0.5mL of naphthalene ethylenediamine hydrochloride solution. Adjust the volume to the mark

with water, mix thoroughly and allow it to stand for 15 minutes.

2) Drawing the Standard Curve: Take 0mL, 2mL, 4mL, 6mL, 8mL, 12mL, 20mL, and 40mL of the sodium nitrite standard solution and place them in 8 (50mL) colorimetric tubes with stoppers. Add 2mL of p-aminobenzene sulfurous solution to each tube and mix well. After standing for 3-5 minutes, add 1mL of naphthalene hydrochloride ethylenediamine solution to each tube. Adjust the volume to the mark with water, mix well, and let it stand for 15 minutes. Use a 1cm cuvette and adjust the zero point with a tube containing 0mL of the standard solution. Measure the absorbance value at a wavelength of 538nm and draw the standard curve.

3) Calculation formula:

$$X = \frac{m_2 \times 1000}{m_3 \times \left(\frac{V_1}{V_0}\right) \times 1000} \tag{5}$$

where: X is the nitrite content in the sample (mg/kg), m_2 is the mass of sodium nitrite in the sample solution (μg), m_3 is the mass of the sample (g), V_1 is the volume of the sample for determination (mL), V_0 is the total volume of the sample treatment solution (mL).

Determination of Superoxide Dismutase Activity (SOD).

The SOD activity was determined by Nitroblue Tetrazolium (NBT) colorimetry method as suggested earlier (Cao Jiankang et al. 2007). The NBT colorimetric method quantifies SOD activity by measuring the inhibition of the reduction of NBT to formazan. The less formazan formed, the higher the SOD activity in the sample. The decrease in color intensity (blue color) is measured using a spectrophotometer at a specific wavelength (usually around 560 nm). The SOD activity is calculated based on the inhibition of NBT reduction.

Determination of Chlorine Dioxide Residue

The residual ClO_2 accumulation was tested using the DPD ultraviolet spectrophotometric method of “National Food Safety Standard Determination of Chlorine Dioxide in Food (2016)” (GB 5009.244-2016), which requires that the surface residue of fruits and vegetables treated with ClO_2 should not exceed 2 mg/kg (5009.244-2016 2016).

Data Analysis

Three different *Toona sinensis* samples were selected in triplicate. 5 chlorine dioxide (ClO_2) treatment groups were used including control and 4 levels. The analysis of variance followed randomized complete block design was utilized. For the data calculation, plotting, origin (2018) software was used (Corporation 2018). The data processing system (DPS; DriverPacks Base) software was used for statistical analysis of difference significance (ANOVA) and then Duncan's new multiple range method test was performed, followed by Tukey's statistical analysis to determine significant differences between groups at 5% level of significance ($P<0.05$).

RESULTS

Weight loss is a key indicator of storage quality in Toon. When toon sprouts are stored in the air, various enzymes accelerate the conversion of sugars into alanine. This alanine then enters a series of tricarboxylic acid cycles, generating heat that leads to water loss. Studies (Jie et al. 2021) have shown that water and dry matter loss during storage period of toon is affected, and water loss and transpiration is a key factor (Jie et al. 2021). The weight loss observed in the Toon from the five treatment groups (Fig. 1) showed an upward trend as storage time increased. The CK group exhibited the highest weight loss, reaching 5.58% by the 8th day, significantly higher than the other treatment groups ($P<0.01$). In contrast, the S3 group had the lowest weight loss, significantly lower than the S1 and S4 groups ($P<0.05$), but not significantly different from the S2 group ($P>0.05$). By the 10th day, the S3 and S2 groups had weight losses of 5.40% and 6.27%, respectively, both significantly lower than the other three groups ($P<0.01$). The results indicate that the concentrations in the S2 and S3 groups could not effectively reduce weight loss and maintain the freshness of Toon. However, chlorine dioxide was shown to slow down the respiration and ultimately delay the wilting of the Toon.

As storage time increased, the appearance of both the CK and experimental groups deteriorated(Fig. 2), with the sensory quality of the toon declining, including loss of color and luster, leaf curling, and eventual wilting. Based on the sensory scores (Fig. 2a), there was no significant difference among the treatment groups during the first 6 days. However, by the day 8, the sensory scores for the CK and S4 groups were significantly lower than those for the S1, S2 and S3 groups ($P<0.01$). The differences between the S1, S2 and S3 groups were not significant ($P>0.05$) on day 8. When storage was extended to the 10th day, the scores for the S2 and S3 groups were not significantly different ($P>0.05$), but they were significantly higher than those for the S1, S4 and CK groups ($P<0.01$). These results demonstrate that the S2 and S3 chlorine dioxide treatment groups could delay the sensory quality deterioration of toon over time. Between days 2 and 8, the degree of leaf wilting in the chlorine dioxide-treated toon buds was significantly lower than in the control group. While chlorine dioxide could not completely inhibit wilting, it significantly slowed the decline in quality of fresh toon buds in the short term.

A decreasing trend reside in total flavonoid activity was observed across the different treatment groups as storage time increased (Fig. 3). This trend may be related to the intensified respiration of toon after harvesting. The S2 group showed the slowest decline in flavonoids, with contents range from 9.20 mg/g, 8.81 mg/g, and 8.44 mg/g on the 4th, 6th, and 8th days, respectively, which were significantly higher than those in the other treatment groups ($P<0.05$). By the 10th day of storage, the CK group had significantly low flavonoid content ($P<0.01$), indicating that chlorine dioxide can slow the depletion of total flavonoid content in Toon, with the S2 group showing the best preservation effect.

Vitamin C (Vc)is an important indicator of freshness in fruits and vegetables due to its ability to effectively remove reactive oxygen species (ROS) (Shuangfang et al. 2020). During the storage period, the vitamin C content in the CK group and other treatment groups significantly decreased from day 0 to day 10, with varying levels of retention in each group (Fig. 4). The CK group had the lowest vitamin C content, which dropped from 42.20 (mg/100g) to 3.94 (mg/100g) by the 10th day, significantly lower than in the S2 and S3 groups ($P<0.05$). The S2 and S3 groups retains the highest vitamin C levels by the 10th day, with a slower decline compared to the CK group, indicating that the chlorine dioxide treatment can delay vitamin C degradation, with the S2 group showing the best preservation effect.

Nitrite levels in food, including Chinese toon (*Toona sinensis*), are crucial for ensuring quality and safety. Nitrites, commonly found in processed meats and preserved foods, pose health risks such as dizziness, poisoning, and even cancer (ShengZhen & ShengZhen 2013). Therefore, monitoring and understanding the dynamic changes in nitrite content during storage is essential to maintaining the quality of Toon. Changes in nitrite content during storage under different treatments (Fig 5), showed that the nitrite levels in all experimental groups were extremely lower than in the CK group ($P<0.01$), though differences among the experimental groups were not significant ($P>0.05$). On the 4th day, chlorine dioxide effectively inhibit nitrite increase. As storage time extended, the S2 and S3 groups had the best inhibitory effect on nitrite, keeping levels within the national food safety standards (⩽40mg/kg), the reason may be that during the storage process of *Toona sinensis*, the microbial activity gradually increases, ultimately increasing the nitrite contents (Xingyu et al. 2019). The CK and S1 groups exhibited significant nitrite peaks on the 8th day, at 186.05 mg/kg and 51.56 mg/kg respectively, both exceeding the national food safety standards (⩽40 mg/kg). Chlorine dioxide concentration of 0.4 ~ 0.8 mg/L significantly reduced nitrite content.

The perishability and decay of fruits and vegetables are closely related to changes in enzymatic activity (Kader 2002). After harvesting, the metabolic activity of fruits and vegetables produces reactive oxygen species (ROS) (Hodges et al. 1999), leading to cell membrane damage and aging (Mittler & Ron 2017). The enzyme SOD can significantly reduce the toxic effects of superoxide anions on cells and regulate the body's oxidative metabolism (Alscher et al. 2002). Results showed that chlorine dioxide concentration of 0.4 and 0.8 mg/L increased SOD enzyme activity, particularly on the 8th and 10th days of storage (Fig 6). On the 8th day, SOD levels were 1288.57 U/g, 1209.61 U/g and 837.19 U/g, in the ClO_2 groups (0.8, 0.4 and 1.6 mg/L respectively), significantly higher than in the CK and 0.2mg/L ClO_2 groups ($P<0.01$). No significant differences were observed on the 2nd, 4th, and 6th days (Fig 6).

Apart from the beneficial impact of ClO_2 on several enzymes and perishability activities, the residual accumulation of chlorine dioxide must be tested to ensure safe food supply to consumer market. Chlorine dioxide quickly decomposes into highly active chlorite and Cl^-, which can easily combine with organic matter to form harmful byproducts. These byproducts can accumulate in the human body and disturbs the body's metabolism, posing serious health risks (Abdel-Rahman et al. 1979). Tests showed that the residual accumulation of chlorine dioxide in Toon decreased gradually with longer storage times (Fig. 7). By the 8th day, the chlorine dioxide residues in the S1 and S2 groups were below 1.75 mg/kg, lower than the national (GB 5009.244-2016) chlorine dioxide residue standard of 2 mg/kg (5009.244-2016 2016). On the 10th day, the chlorine dioxide residues in the S2 and S3 groups remained below the national standard, with residues of 1.60 mg/kg and 1.65 mg/kg, respectively.

DISCUSSION

Chlorine dioxide is an important disinfectant categorized as A1 and is well known for its adaptive features. Apart from its beneficial aspects, a detailed study was needed to access its impact on various biochemical and residual effects of *Toona sinensis*. The weight loss, sensory evaluation, quality gradient, total flavonoid, vitamin C, nitrite concentration, SOD activity and residual accumulation in Toon samples were estimated to provide a complete detailed study for long term preservation and post-harvest storage of quality products. This study presents compelling evidence on the effectiveness of chlorine dioxide (ClO_2) treatments in maintaining the quality and extending the shelf life of *Toona sinensis* during storage. The post-harvest ClO_2 treatment can slow down the respiration of Toon and effectively reduce the weight loss (Fengyuan et al. 2017). The sensory analysis further supports the efficacy of ClO_2 in preserving Toon freshness. The sensory quality index under different ClO_2 treatment levels (Fig 2b) disclosed that chlorine dioxide can delay visual and textural degradation (Karabulut et al. 2009). It can effectively inhibit the decay of Toon and maintain good sensory quality (average around 80 points). One of the possible reason is the chlorine dioxide's ability to destroy the stability of bacterial cell membranes, inhibit the synthesis of microbial proteins, effectively destroy microorganisms, improve the antioxidant capacity and disease resistance of Toon, and reduce the consumption of nutrients (Xiaoyu 2006).

The results also indicate that chlorine dioxide can effectively slow the depletion of bioactive compounds such as total flavonoid content and vitamin C. Appropriately, the chlorine dioxide treatment of 0.4 mg/L had the high impact for retention of flavonoids and vitamin C in Toon by day 10. Flavonoids have biological activities such as anti-cancer, antioxidant, anti-aging, anti-inflammatory, and immunity-boosting. Vitamin C is naturally reducible in products (Aldhaher & H 2016; Peng et al. 2019). Fang and colleagues found that chlorine dioxide has strong oxidizing property, and its reducing effects on various compounds, including Vitamin C, during the treatment of fresh produce (Fang et al. 2012). The results show that the balanced concentration of chlorine dioxide, appropriate treatment duration and suitable storage conditions can effectively delay the degradation of vitamin C by controlling microbial growth and reducing oxidative stress on the produce (Fang et al. 2012).

Nitrite can be naturally present in food or can be added as a preservative, and its concentration is regulated due to its potential health effects. The chlorine dioxide had a significant inhibitory effect on the nitrite content especially on the 4th day of treatment application. As storage time increase, a significant nitrous peak reduction appeared on the 8th and 10th day, indicating ClO_2 inhibitory effect. This difference in effectiveness may be attributed to the strong oxidizing properties of chlorine dioxide, which can lead to the destruction of the cell structure of *Toona sinensis* at higher concentrations. The treatment (0.4 and 0.8 mg/L) effectively maintained the quality of *Toona sinensis*. The chlorine dioxide high concentration during the early stages might be a reason, which inhibit the increase of nitrite content. The ClO_2 treatment can help mitigate this risk by reducing nitrite levels while still providing effective antimicrobial action and subsequent nitrite formation as indicated by Zhong and Zhang (Zhong & Zhang 2013). The reduction of nitrite content through ClO_2 treatment can be advantageous in food preservation as it can inhibit the growth of nitrite-producing bacteria, thus preventing the accumulation of harmful levels of nitrites in stored foods. Especially in products where controlling nitrite levels is important for safety and regulatory compliance.

The degradation of enzymatic activity results in depletion of perishability in food products. Superoxide dismutase (SOD) is the main enzyme that scavenges ROS and plays a critical role in protecting cells from oxidative damage. The level of ROS scavenging enzymes is closely related to the storage stability and quality attributes in Chinese toon (Lin et al. 2020; Lin et al. 2017). The SOD activity on the 8th day for 0.8 and 0.4 mg/L ClO_2 treatment group was significantly higher. The changes on 2nd, 4th, and 6th day were non-significant. The 0.4 and 0.8 mg/L concentration of chlorine dioxide improves the activity of SOD enzymes and helps in alleviating the perishability of *Toona sinensis*. The increased SOD activity suggests that ClO_2 can bloster the antioxidant defense of the Toon, thereby extending its shelf life. This is consistent with the findings of karabulut, who reported that ClO_2 treatments could enhance the antioxidant enzyme activities in fresh produce, contributing to reduced oxidative stress and prolonged freshness (Karabulut et al. 2009). The improvement in SOD activity of leaves due to ClO_2 treatment was also observed by Qiwei et al. 2015 (Qiwei et al. 2015).

However, the potential for ClO_2 residue accumulation must be carefully monitored to ensure consumer safety. The findings of current experiment disclosed that ClO_2 residue in the S2 and S3 groups were within safe limit by the 10^{th} day of storage, suggesting its safe and effective use. This is crucial as ClO_2 can decompose into by-products that, if accumulated in significant quantities, could pose health risks (Abdel-Rahman et al. 1979). The high concentration of chlorine dioxide residues in plant tissues is attributed to several factors (Li & Huang 2008). Some residues may remain on the surface of the leaves or within the plant tissue, can accumulate if not adequately removed during subsequent processing/washing steps (Gómez-López et al. 2009). Therefore, proper dosage, optimal treatment duration, effective rinsing/washing and monitoring of samples should be an important practice to ensure effective reduction of harmful components in Toon without compromising its product quality or safety.

CONCLUSIONS

The results of this study indicated that the chlorine dioxide treatment at concentrations of 0.4 mg/L and 0.8 mg/L were better compared to concentrations of 1.6 mg/L and 0.2 mg/L. Key findings indicate that different ClO_2 treatments significantly affect the sensory attributes of Toona sinensis, with implications for improving its storage quality. It can effectively mitigate weight loss, delay sensory deterioration, and preserve key nutritional components such as flavonoids and vitamin C for more than 8 days. Additionally, ClO_2 was effective in reducing nitrite accumulation, and enhancing the activity of superoxide dismutase, which is critical for food safety. Adhering to the National standards for chlorine dioxide residue (<2.0 mg/kg). Overall, the study demonstrates that appropriate chlorine dioxide concentration of 0.4 and 0.8 mg/L is an effective treatment for preserving the quality and extending the shelf life (for 8 days) of *Toona sinensis* under the conditions of 20±2°C, improving the post-harvest storage.

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Authorship Contribution Statement

RZ conceived the project and designed the experiments; RZ, YG and MZ performed the experiments; RZ, MUF and YJ analyzed the data; RZ, MUF and JC were major contributors in writing the manuscript; RZ, KMAA and MAF give the funding acquisition; MUF, ML and YP review and editing the manuscript; all authors discussed the results and reviewed the manuscript.

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Data Availability Statement

The data associated with this study was not deposited into a publicly available repository before. All data are included in the manuscript.

Competing interests

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.

Complete Ethical statement

Not Applicable

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Figure 1

Effect of different chlorine dioxide (ClO_2) treatments on weight loss of *Toona sinensis*

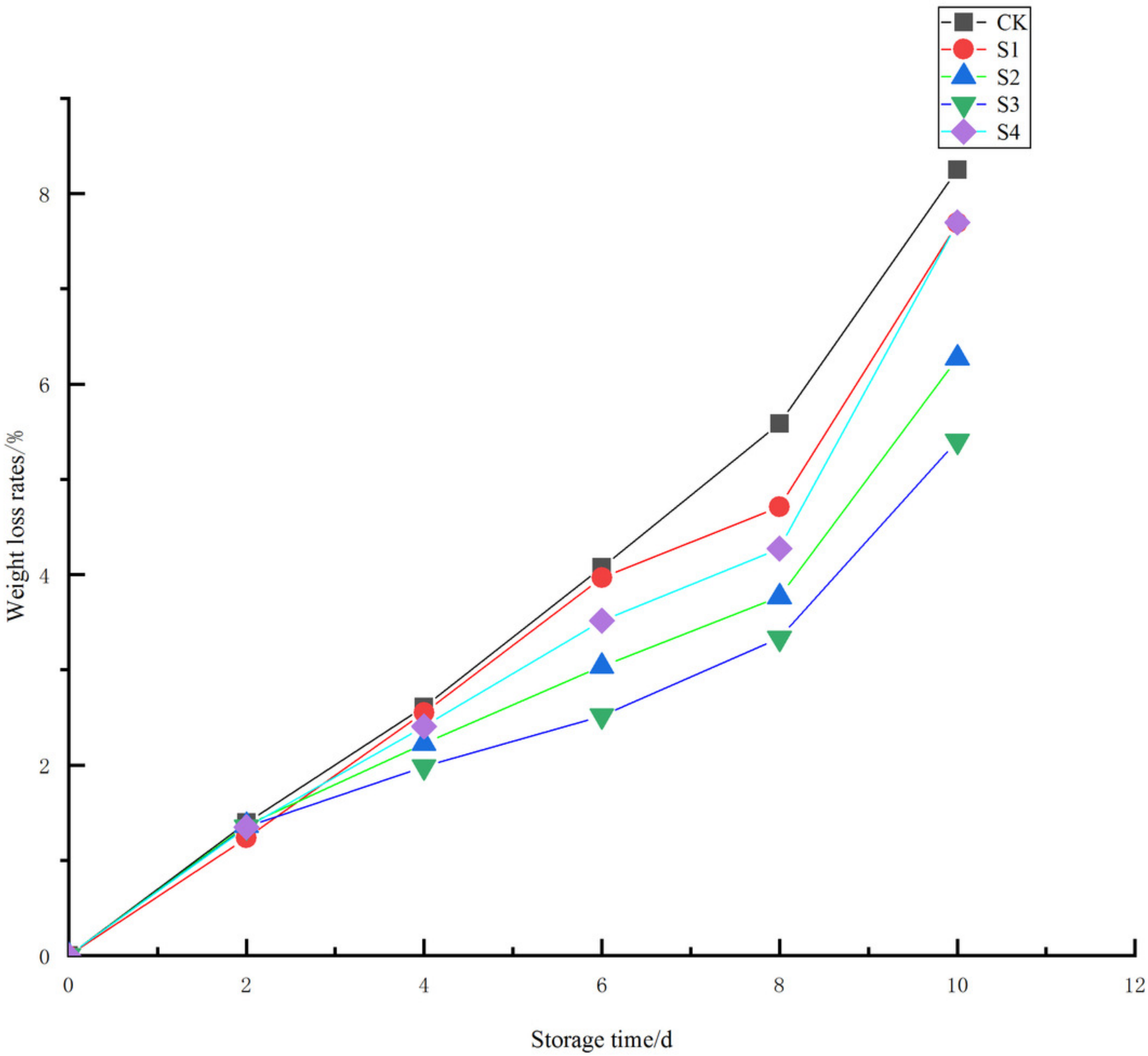
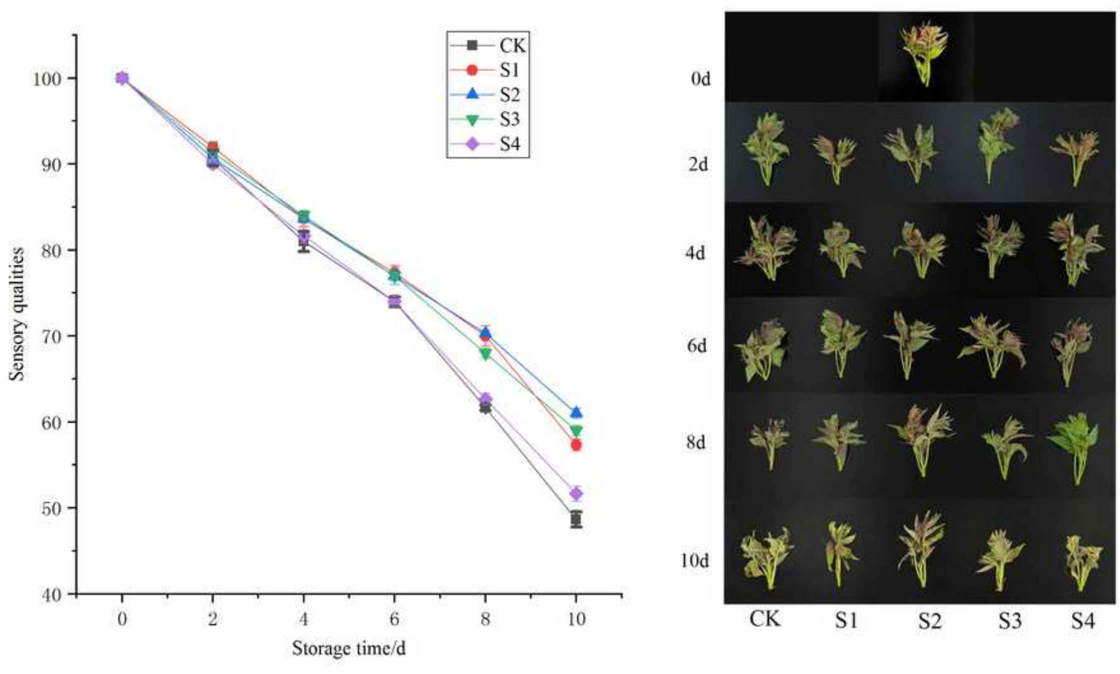


Figure 2

Fig.2 Effect of different chlorine dioxide treatments on sensory evaluation of *Toona sinensis*

Note: (a) is the sensory score of different chlorine dioxide treatments on Toons; (b) is the appearance of Toon due to different chlorine dioxide treatments.



(a) (b)

Figure 3

Fig.3 Effects of different chlorine dioxide treatments on the total flavonoid content of *Toona sinensis*

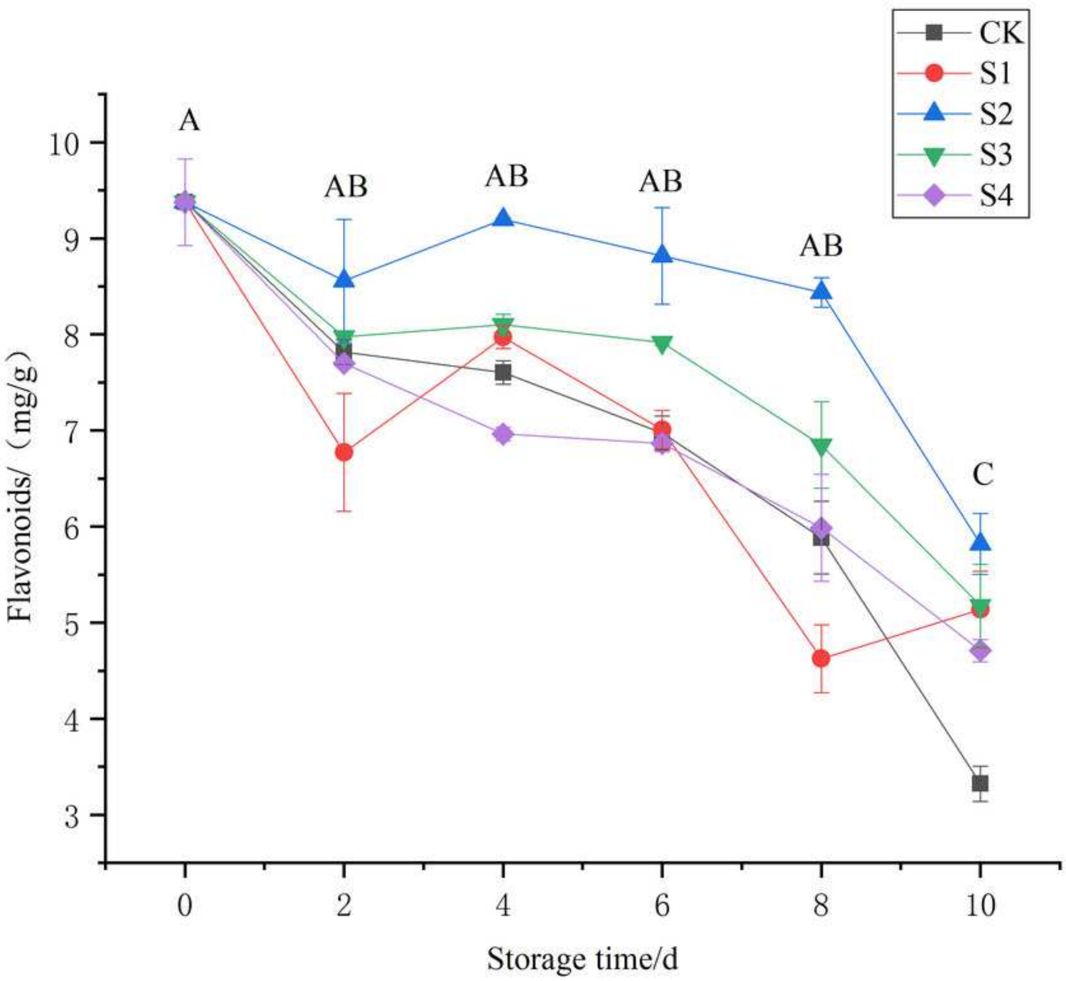


Figure 4

Fig.4 Effect of different chlorine dioxide treatments on the Vitamin C content of *Toona sinensis*

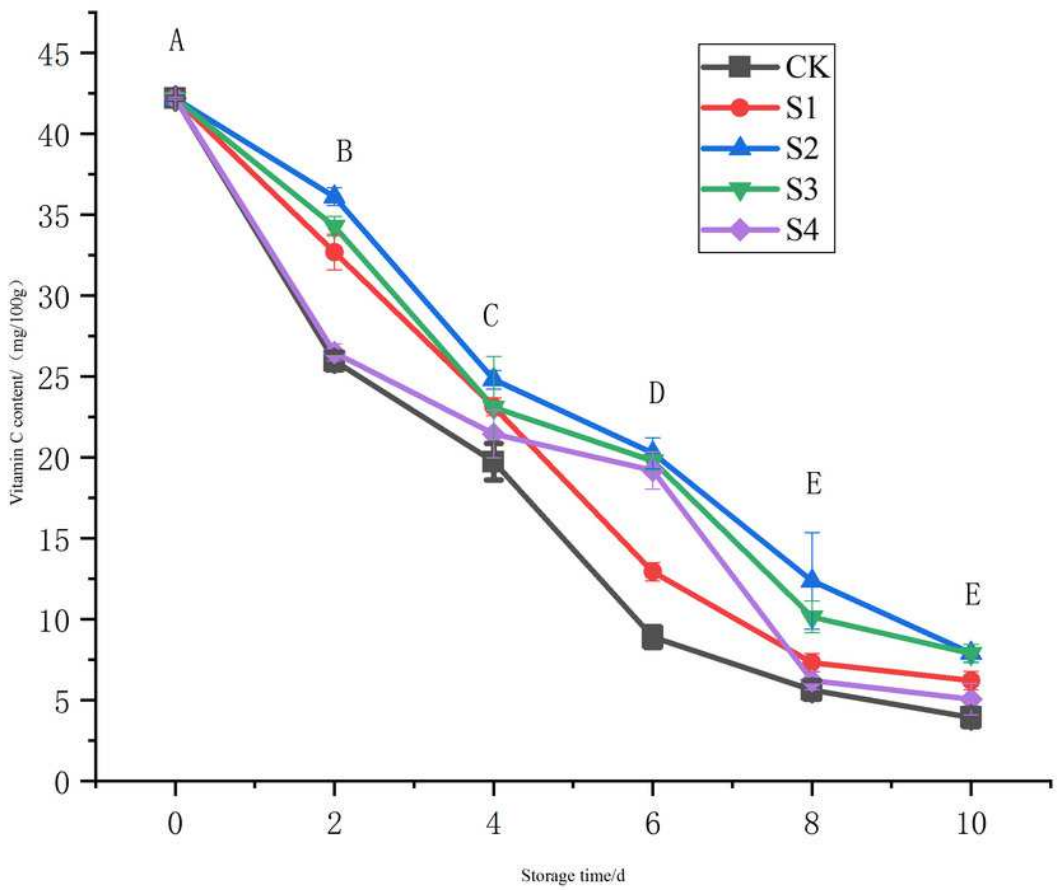


Figure 5

Effect of different chlorine dioxide treatments on nitrite concentration of *Toona sinensis*

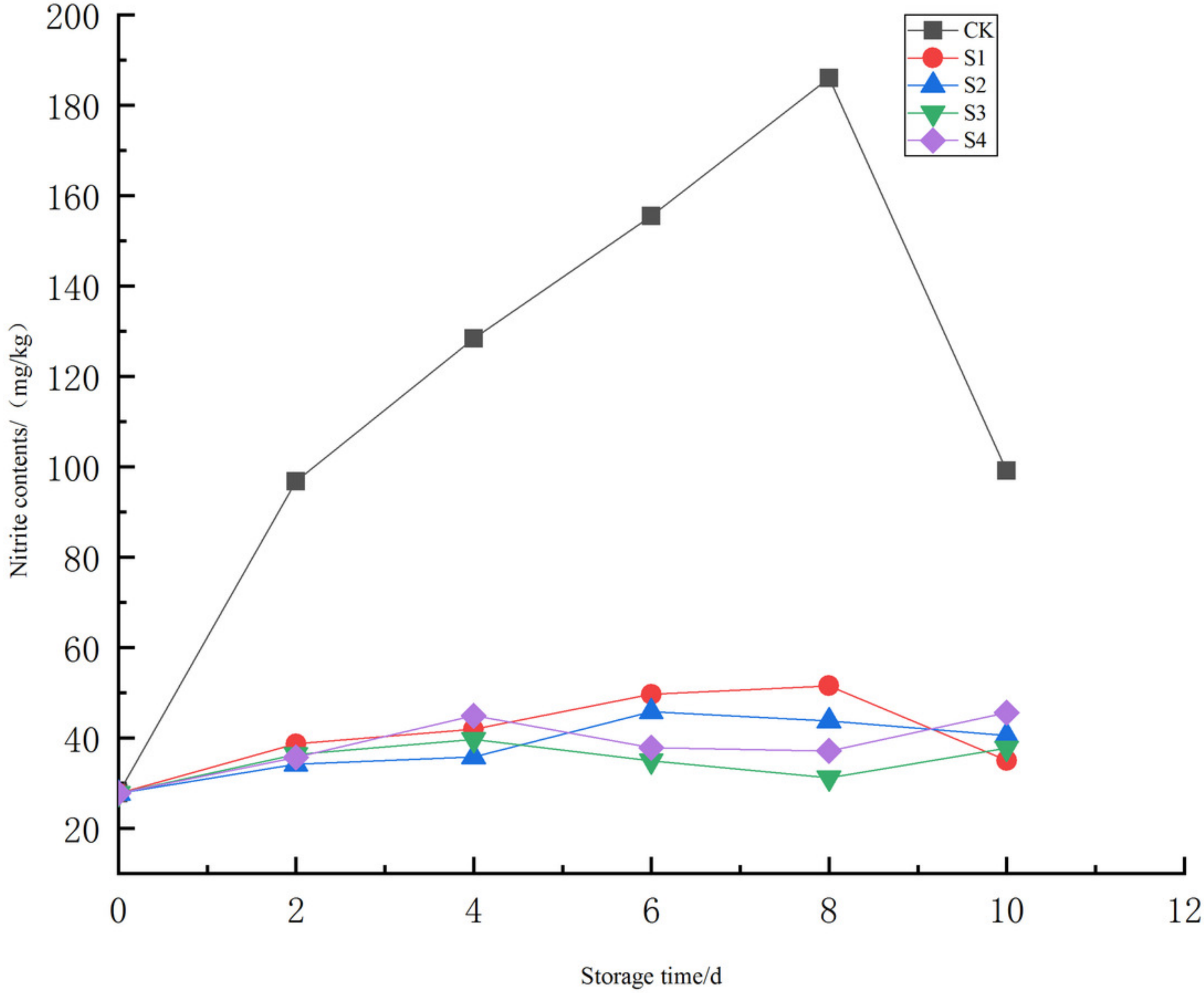


Figure 6

Fig.6 Effects of different chlorine dioxide treatments on SOD enzyme content of *Toona sinensis*

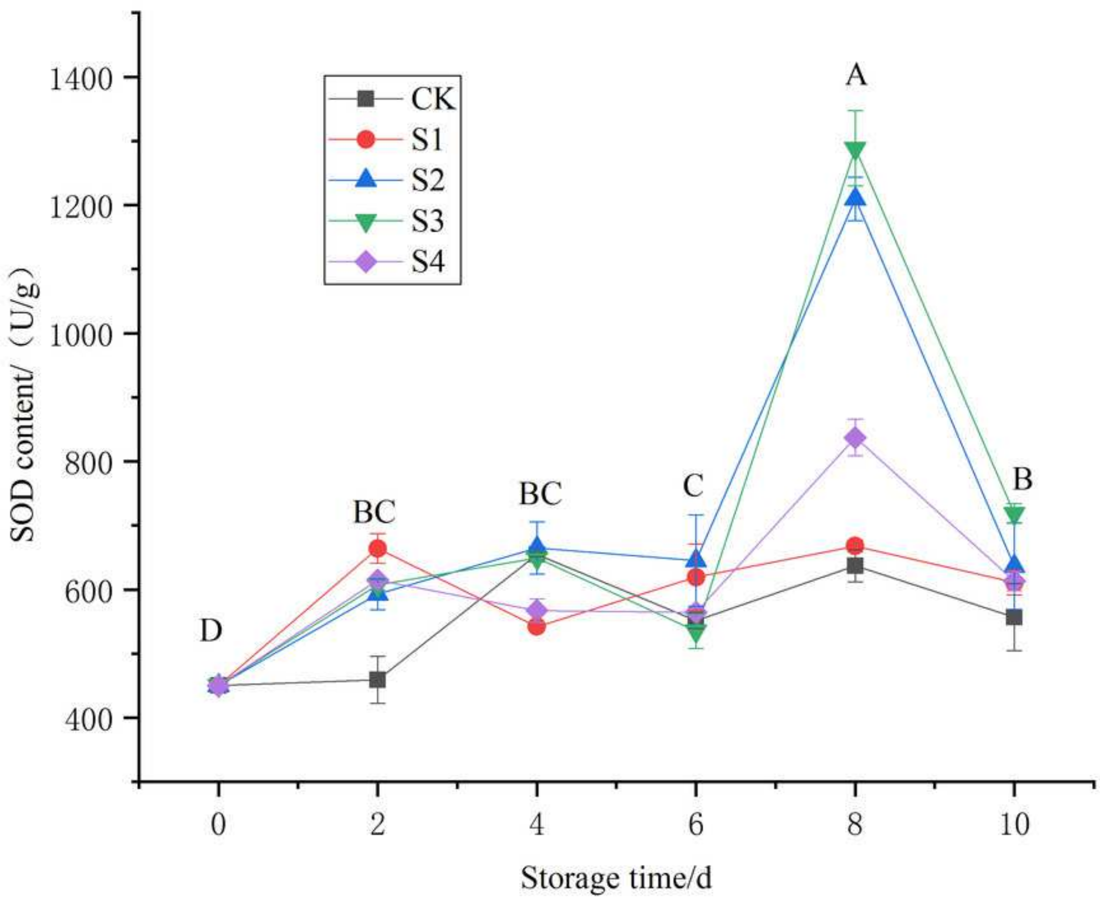


Figure 7

Fig.7 Chlorine dioxide residues in *Toona sinensis* under different chlorine dioxide treatments