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Pre-Harvest Application of Multi-Walled Carbon Nanotubes Improves the Antioxidant Capacity of ‘Flame Seedless’ Grapes during Storage

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Abstract: As a widely distributed fruit, grapes are susceptible to oxidative damage during storage and transportation, resulting in declining quality and commodity value. This study aimed to investigate the effects of preharvest application of different concentrations of multi-walled carbon nanotubes (MWCNTs) on the postharvest quality of ‘Flame Seedless’ grapes. The results showed that low-concentration (25 and 50 mg L^{−1}) MWCNTs treatments maintained the comprehensive quality index, firmness, soluble sugar, titratable acid, pH value, and ascorbic acid (AsA) content of grapes. MWCNTs at 25 and 50 mg L^{−1} increased the activities of peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), and ascorbic acid (APX). Furthermore, MWCNTs reduced the malondialdehyde (MDA) content and decreased the accumulation of excessive reactive oxygen species (ROS) in grape peel and pulp tissues. In addition, transmission electron microscopy (TEM) images demonstrated that MWCNTs were absorbed by parenchymal cells in the grape peel and pulp through the epidermal cell layer. MWCNTs with a specific concentration can be used as a new inducer for the biosynthesis of antioxidants to reduce oxidative damage in grapes during storage.

Keywords: table grape; multi-walled carbon nanotubes; OXIDATIVE damage



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1. Introduction

The grape is a berry fruit of the genus *Vitis* L. in the family Vitaceae. It is native to Europe and western Asia and is mostly found in temperate and subtropical zones. The grape is one of the earliest cultivated and most widely distributed fruit globally, with more than 5000 years of cultivation history.

Grape is a non-climacteric fruit with relatively low physiological activity. So far, the most commonly used methods are physical treatments and natural or artificial compounds treatments, so as to reduce the decline in quality of postharvest grapes [1,2]. One of the most widely used methods in the world is fumigation with SO₂ release pads or sulfur dioxide (SO₂) [3–5]. However, SO₂ also causes adverse changes in the taste, aroma, and color of grape berries [5] and environmental problems, and adversely affects human health. For these reasons, there is a growing tendency to use healthy and eco-friendly alternatives to preserve the valuable internal ingredients of grapes after harvest [6]. In recent years, using nanomaterials for post-harvest preservation has attracted extensive attention globally because of its advantages, such as solid adaptability, low cost, and long preservation period [7]. It has also been applied to preserving various fruits and vegetables and achieved good results [8]. Studies have demonstrated that chitosan nanoparticles delay the ripening process of grapes, decrease the weight loss rate and soluble solids content, reduce the sugar content, and enhance the retention of titratable acidity and sensory properties [9].

Multi-walled carbon nanotubes (MWCNTs) are one of the most commonly used nano-materials globally [10]. Due to their unique mechanical and physical chemical properties, multi-walled carbon nanotubes have been used as potential solutions for different biologically important problems [11]. The use of multi-walled carbon nanotubes in food science has raised concerns about their biological impact [12]. Previous studies have shown that MWCNTs alter metabolic, biochemical, and physiological processes in plants including the seed germination rate, biomass accumulation including root and stem length, and activating cell division [13–19]. In addition, several studies have shown that the multiple effects of MWCNTs on plants exhibit a concentration-dependent form pattern. Low concentrations of MWCNTs can promote seedling growth and bio-yield of *Cucurbita pepo* L. [20] and tomato. Ingested and infiltrated by cells, MWCNTs enhance the antioxidant capacity by activating antioxidant enzymes such as SOD, POD, and CAT, and producing non-enzymatic antioxidants such as ascorbic acid [21]. MWCNTs can decrease ROS and malondialdehyde accumulation by activating antioxidant systems in plants [22]. MWCNTs can enhance the antioxidant capacity of the medicinal plant *Khuzestanica* cultured in vitro [10,23]. Previous studies on MWCNTs focused on their improvement of antioxidant capacity in plant roots, stems, and leaves, and few on their effect on fruit preservation. Improving antioxidant capacity and alleviating oxidative damage are vital for prolonging the storage period of grapes [24]. However, there is no systematic report on the preservation of grapes by MWCNTs.

This study attempts to investigate the effects of spraying different concentrations of MWCNTs before harvest on fruit quality, ROS production, and activities of various antioxidant enzymes of ‘Flame Seedless’ grapes during postharvest storage. This study also explores the mechanism of MWCNTs in reducing oxidative damage of table grapes through ROS metabolism. Transmission electron microscopy (TEM) images are used to observe the action pathway of MWCNTs. This study provides technical support for the application of MWCNTs, a new material with great potential in the preservation of fruits and vegetables.

2. Materials and Methods

2.1. Preparation of MWCNTs

MWCNTs (purity > 95%) were purchased from Chengdu Organic Chemicals Co., Ltd., Chengdu, China, Chinese Academy of Sciences. MWCNTs powder was directly suspended in distilled water and dispersed for 20 min under the action of a 35 kHz ultrasonic signal to obtain a colloidal suspension. MWCNTs with different concentrations (0, 25 50, and 100 mg L⁻¹) were prepared, and the resulting homogenized colloidal suspension was stored at 4 °C and used for in vitro experiments within 1 month after preparation.

Characterization of MWCNTs

The MWCNTs were characterized qualitatively and quantitatively with TEM and SEM images (Figure 1A,B) and TGA (Figure 1C). The outer diameter of MWCNTs was 5–15 nm, the length was 10–30 µm, the purity was over 95%, the ash content was below 3% wt, and the specific surface area was 220–300 m² g⁻¹.

2.2. Plant Materials and Treatment

In this study, the ‘Flame Seedless’ grape of 2021 was used as the experimental material, and grape plants (annual cutting seedlings) were planted in the Grape Standard Test Garden of the Xinjiang Shihezi Academy of Agricultural and Reclamation Sciences in 2015 (lat. 44°59′ N, long. 86°02′ E). The row spacing was 3.0 m, the plant spacing was 1.0 m, and drip irrigation was used for topdressing and irrigation. The tested grapes sprouted on 29 March 2021, bloomed on 22 May, and reached their full bloom on 28 May. In the experiment, a randomized block design was adopted. A single grapevine was the experimental unit, and 5 replicates were set. The grapevines chosen for the test were of similar size and fruit-bearing capacity, with approximately 30–40 fruit-bearing branches. A

control treatment (CK) and three MWCNTs concentration treatments of 25 mg L⁻¹ (M1), 50 mg L⁻¹ (M2), and 100 mg L⁻¹ (M3) were set up in this experiment at the ear stage (15 days after flowering), the fruit expansion stage (40 days after flowering), and the veraison (60 days after flowering). The MWCNTs were sprayed directly on the whole grape plant at 4 L/plant, and the control group was sprayed with the same amount of water.

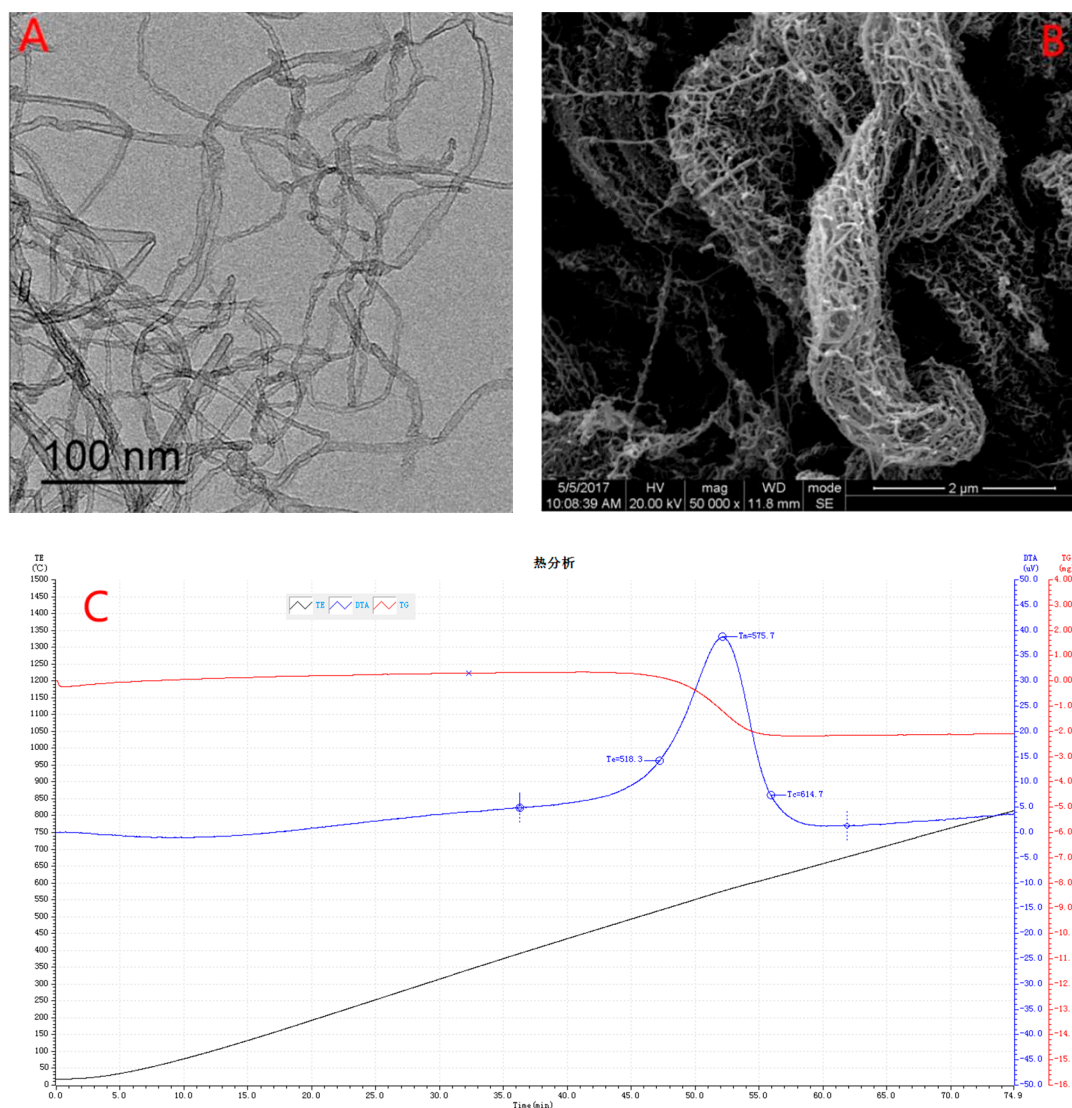


Figure 1. Properties of multi-walled carbon nanotubes. (A,B) TEM and SEM images; (C) TGA.

2.2.1. Postharvest Treatment

When ‘Flame Seedless’ grapes reached commercial maturity (SSC of $20 \pm 1\%$), fully ripe grape clusters were randomly picked near and away from the root (Figure 2) and transported to the laboratory. Grape bunches with the same size, color, shape, and no mechanical pathological damage were selected for the determination of soluble solids, firmness, and pH value within 1 h. The remaining grapes were packed and marked according to the treatment methods. The grapes were stored at 0 °C for 12 h to remove field heat. After the pretreatment, all samples were stored in a 4 °C refrigerator for 20 d. After each storage interval (every 5 d), the physicochemical properties of the fruit were assessed. Approximately 400 g of grapes were selected as replicates from the top, middle, and bottom of the bunches of the different treatments, with 5 biological replicates. Then 50 samples of grapes from each replicate were randomly selected for quality analysis until the end of the storage period.

2.2.2. Grape Photos Taken at Maturity

Grape clusters of the shape in Figure 2 were selected as experimental material for harvesting.



Figure 2. “Flame Seedless” during harvest. The concentrations of (A–D) sprayed multi-walled carbon nanotubes were 25, 50, 100, and 0 mg L^{−1}, respectively.

2.3. Determination items and Methods

2.3.1. Physiochemical Properties of Grapes

Soluble solids content (SSC) and titratable acidity were measured with a Brix-acid dual scale digital refractometer (PAL-BX-ACID91, Atago Co., Ltd., Tokyo, Japan) at 25 °C according to the manufacturer’s instructions. TA was determined by titrating 10 mL of juice to pH 8.2 with 0.02 mol L^{−1} NaOH and expressed as grams of tartaric acid equivalent per 100 mL of juice. The pH value of the juice was determined by direct immersion of the electrode (Hanna Instruments Inc., Bucharest, Romania). Ascorbic acid (AsA) content was determined by titration with 2, 6-dichloroindophenol sodium salt hydrate, and expressed as mg kg^{−1} on a fresh weight basis. The firmness was measured by a GY-4 hardness tester (Leqing Edbao Instrument Co., Ltd., Beijing, China) with a probe diameter at 5.0 mm and expressed as N.

2.3.2. Analysis of Malondialdehyde, H₂O₂ and O₂[−] Production Rate

MDA content was measured regarding the method of Xu et al. [25]. We weighed 1 g each of the cut frozen peel and pulp tissue, added 2 mL of 10% TCA and a small amount of quartz sand, ground them until homogenized, then added 8 mL of the TCA and ground it. The homogenate was centrifuged at 4000 × g for 10 min, and the supernatant was the sample extract. We aspirated 2 mL of the supernatant of the centrifugation, added 2 mL of the 0.6% TBA solution, and the homogenate was reacted in a boiling water bath for 15 min and then centrifuged after rapid cooling. The absorbance values at 532, 600, and 450 nm were determined by taking the supernatant and expressing it as μmol kg^{−1} (based on fresh weight).

The peel and pulp powders (0.2 g) were mixed with 2 mL of the 65 mmol L^{−1} PBS (pH 7.8) for grinding, and then centrifuged for 20 min (12,000 × g, 4 °C). The supernatant was collected to determine the O₂[−] content according to the instructions of the O₂[−] kit (Solarbio Science & Technology Co., Ltd., Beijing, China). The O₂[−] content was expressed as mmol min^{−1} kg^{−1} (based on fresh weight).

The peel and pulp powders (0.2 g) were mixed with 2 mL of pre-cooled acetone and centrifuged for 10 min (8000 × g, 4 °C). The supernatants were collected to determine the H₂O₂ content according to the instructions of the H₂O₂ kit (Solarbio Science & Technology Co., Ltd., Beijing, China). The H₂O₂ content was expressed as mmol kg^{−1} (based on fresh weight).

2.3.3. Assay of Antioxidant Enzyme Activity Content

The peel lyophilized powder (0.5 g) and 5 g of pulp lyophilized powder were homogenized by adding 5 mL and 50 mL of 0.05 mol L⁻¹ phosphate buffer (pH 7.8, containing 0.1 mmol L⁻¹ EDTA), respectively, and centrifuged at 12,000× g for 20 min at 4 °C. The supernatant was collected.

POD activity was measured as described previously by Wang et al. [26]. The reaction solution contained 1700 µL of 25 mmol L⁻¹ PBS (pH 7.0, containing 0.1 mmol L⁻¹ EDTA), 100 µL of 1% guaiacol, 100 µL of 20 mmol L⁻¹ H₂O₂, and 100 µL of supernatant. The absorbance at 470 nm was measured. One unit (U) of POD activity was defined as an absorbance increase of 0.01 in 1 min, and POD activity was expressed as U mg⁻¹ (in protein).

CAT activity was measured as described previously by Zheng et al. [27]. The reaction solution contained 1700 µL of 25 mmol L⁻¹ PBS (pH 7.0, containing 0.1 mmol L⁻¹ EDTA), 200 µL of 100 mmol L⁻¹ H₂O₂, and 100 µL of the supernatant. The absorbance at 240 nm was measured. One unit (U) of CAT activity was defined as an absorbance increase of 0.01 in 1 min, and CAT activity was expressed as U mg⁻¹ (in protein).

SOD activity was measured as described previously by Zheng et al. [27]. The reaction solution contained 50 µL of the supernatant and 3 mL of 50 mmol L⁻¹ phosphate buffer (pH 7.8, containing 130 mmol L⁻¹ of methionine, 750 µmol L⁻¹ of NBT, 20 µmol L⁻¹ of riboflavin, 0.5 mmol L⁻¹ EDTA). The reaction solution was incubated at 25 °C, 4000 lx, for 20 min. The absorbance at 560 nm was measured. One unit (U) of SOD activity was defined as the amount of enzyme required to inhibit the reduction of NBT to half (50%), and SOD activity was expressed as U mg⁻¹ (in protein).

APX activity was measured as described previously by Li et al. [28]. The reaction solution contained 1700 µL of 25 mmol L⁻¹ PBS (pH 7.0, containing 0.1 mmol L⁻¹ EDTA), 100 µL of 20 mmol L⁻¹ H₂O₂, 100 µL of 5 mmol L⁻¹ AsA, and 100 µL of the supernatant. The absorbance at 290 nm was measured. One unit (U) of APX activity was defined as an absorbance increase of 0.01 in 1 min, and APX activity was expressed as U mg⁻¹ (in protein).

Protein content was determined according to Bradford [29].

2.3.4. Morphological Observation by TEM

The grape peel and pulp on the day of harvest were further observed by TEM to examine whether pristine MWCNTs entered the plant cells. The grape peel and pulp samples were cut open. Then, according to Lin et al. [30], the ultrathin sections were mounted on copper grinds to take TEM images.

2.3.5. Statistical Analysis

All statistical analyses were performed with a one-way analysis of variance (ANOVA) using SPSS 26.0 (SPSS Inc., Chicago, IL, USA). The LSD multiple-range test was used for comparison between groups. Tukey's test was adopted for calculating the differences between means at a significance level of 5%, and Origin 2021 was used for graphing.

3. Results

3.1. Biochemical and Physical Quality Characteristics of Grape in Storage

As shown in Table 1, MWCNTs treatment positively affected the SSC, TA, and pH values of grapes during storage. Specifically, SSC showed a gradual upward trend on the 20th day of the storage period. On the 20th day, the SSC content in the M1 treatment was the highest, while that in the control was the lowest. TA gradually decreased during the storage period, but the difference among the treatments was not noticeable. The pH value of grapes increased slightly during the storage period, and the CK treatment had the highest pH value (3.83%) on the 20th day. During the storage period, AsA and firmness showed a trend of first increasing and then decreasing. AsA peaked on day 15, and firmness

peaked on day 5. The AsA and firmness in the M2 treatment were higher than those in the control group by 24.58% and 26.23%, respectively.

Table 1. Evolution of bio-chemical properties of grapes treated with MWCNTs during storage.

Postharvest	Storage Time (Day)				
Quality	0	5	10	15	20
SSC (%)					
M1	21.43 ± 0.12 a	21.87 ± 0.58 c	21.93 ± 0.58 b	22.50 ± 0.20 b	23.70 ± 0.10 a
M2	21.23 ± 0.21 ab	22.70 ± 0.10 a	22.07 ± 0.58 ab	23.20 ± 0.17 a	23.43 ± 0.11 b
M3	21.10 ± 0.12 b	22.30 ± 0.10 b	22.37 ± 0.58 a	22.40 ± 0.10 b	23.07 ± 0.06 c
CK	20.03 ± 0.06 c	20.53 ± 0.11 d	21.43 ± 0.32 c	21.60 ± 0.10 c	22.10 ± 0.10 d
TA (%)					
M1	0.59 ± 0.03 a	0.43 ± 0.02 a	0.36 ± 0.01 a	0.34 ± 0.01 b	0.29 ± 0.01 a
M2	0.54 ± 0.02 ab	0.45 ± 0.07 a	0.43 ± 0.01 a	0.41 ± 0.04 a	0.25 ± 0.01 a
M3	0.59 ± 0.05 a	0.46 ± 0.06 a	0.39 ± 0.02 a	0.36 ± 0.04 ab	0.33 ± 0.03 a
CK	0.51 ± 0.01 b	0.41 ± 0.01 a	0.40 ± 0.02 a	0.37 ± 0.03 ab	0.30 ± 0.07 a
pH					
M1	3.37 ± 0.04 c	3.43 ± 0.02 c	3.56 ± 0.01 b	3.58 ± 0.01 c	3.75 ± 0.01 b
M2	3.43 ± 0.01 b	3.46 ± 0.01 b	3.52 ± 0.01 a	3.55 ± 0.01 c	3.76 ± 0.02 b
M3	3.48 ± 0.01 ab	3.49 ± 0.01 a	3.56 ± 0.01 b	3.62 ± 0.02 b	3.71 ± 0.01 c
CK	3.49 ± 0.01 a	3.46 ± 0.01 b	3.60 ± 0.02 a	3.75 ± 0.01 a	3.83 ± 0.01 a
AsA (mg/kg)					
M1	4.75 ± 0.49 a	12.29 ± 1.23 a	13.92 ± 4.09 b	15.91 ± 0.30 bc	6.75 ± 0.12 a
M2	6.75 ± 0.59 a	12.5 ± 0.90 a	21.38 ± 1.04 a	28.22 ± 8.18 a	10.18 ± 0.10 a
M3	7.94 ± 0.67 a	9.71 ± 2.27 a	11.69 ± 2.76 b	25.39 ± 5.09 ab	11.06 ± 1.27 a
CK	6.52 ± 0.10 a	11.69 ± 0.79 a	11.57 ± 1.57 b	12.00 ± 3.29 c	10.17 ± 1.74 a
Firmness (N)					
M1	3.93 ± 0.23 bc	5.87 ± 0.32 b	5.83 ± 0.32 a	5.20 ± 0.20 a	4.37 ± 0.15 a
M2	4.50 ± 0.35 a	6.40 ± 0.10 a	5.13 ± 0.21 b	5.00 ± 0.17 a	4.33 ± 0.32 a
M3	4.37 ± 0.15 ab	6.20 ± 0.10 ab	5.40 ± 0.17 ab	4.33 ± 0.15 b	3.70 ± 0.72 ab
CK	3.80 ± 0.26 c	5.07 ± 0.11 c	5.60 ± 0.10 a	4.10 ± 0.17 b	3.17 ± 0.11 b

Different letters indicate significant differences ($p < 0.05$ by LSD test). All values are expressed as the mean ± SE of three replicates.

3.2. Production of MDA, O_2^- , and H_2O_2 during Storage

The malondialdehyde (MDA) content in grape peel and pulp showed a similar rule. The MDA content of both control and MWCNTs-treated grapes continued to increase during storage (Figure 3A,B). On the 10th day of storage, the content of M3 treatment MDA in peel was much higher than that of other treatments and 2.43 times that of the control group. Nevertheless, the content of MDA in peel was no significant difference between the control treatment and other treatments on other storage days. The MDA content in the pulp of the control treatment was higher than that of the other three treatments, especially on the 15th day and the 20th day. The MDA content of the M2 treatment remained the lowest from the 10th day to the 20th day, measured at 4.49 and 4.27 $\mu\text{mol kg}^{-1}$, respectively.

The O_2^- production rates in the peel and pulp tissues are shown in Figure 3C,D. The O_2^- production rate in peel and pulp rose gradually with increasing storage time. In the peel, the O_2^- production rate of the M1 treatment was low. On the 15th day of storage, the O_2^- production rate of the M1 treatment was lower than that of other treatments and was 33.06% lower than that of the control treatment. At the end of the storage period, the O_2^- production rate of the M2 treatment decreased sharply, which was 34.22% lower than that of the control treatment. In the pulp, the O_2^- production rate of the M1 treatment increased slowly. At the end of the storage period, the O_2^- production rate of the control treatment was higher than that of all other treatments, which were 1.64, 1.77, and 1.60 times that of M1, M2, and M3, respectively.

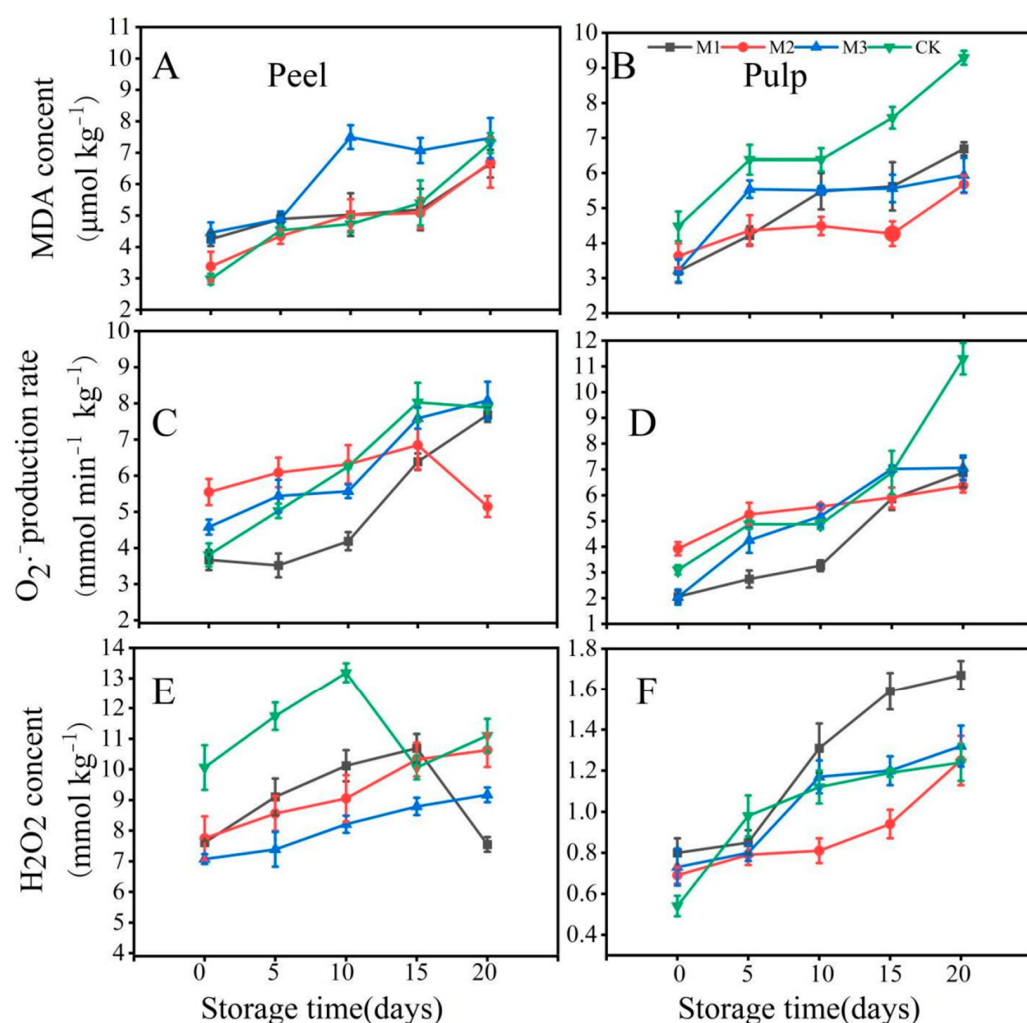


Figure 3. MDA (A,B), O_2^- (C,D), and H_2O_2 (E,F) content production in the peel and pulp tissues of 'Flame Seedless' grape after MWCNT treatment. Data are means of three replicates; error bars represent SE.

The H_2O_2 content in peel and pulp showed different variation trends (Figure 3E,F). In the peel tissue, the M3 treatment reduced the H_2O_2 content. In the first 10 days of the storage period, the H_2O_2 content of the control treatment was higher than that of the other three treatments, and the maximum was 1.30, 1.70, and 1.60 times that of the M1, M2, and M3 treatments, respectively. The peel had a higher H_2O_2 content than the pulp. There was no significant difference between each treatment and the control in pulp in the first 5 days of the storage period. However, on the 10th and 15th days of storage, the H_2O_2 content of the M2 treatment was lower than that of the control treatment, reaching approximately 72.24% and 57.98% of the control treatment, respectively. The H_2O_2 content of the M1 treatment increased sharply from the 10th day and peaked at $1.67 \text{ mmol kg}^{-1}$ on the 20th day.

3.3. Antioxidant Enzyme Analysis

POD activity in the peels of all treated grapes peaked at day 5 (Figure 4A), among which the M2 treatment had the highest POD activity, 1.54 times that of the control treatment. On the 15th day of storage, the POD activity of the M2 treatment was 28.96% higher than that of the control treatment. The POD activity in the pulp also peaked on the 5th day (Figure 4B), and the POD activity of the M2 treatment was higher than (1.99 times) that of the control treatment. At the end of the storage period, the POD activity of the M3 treatment was higher than that of other treatments, which was 50.84% higher than that of the control treatment.

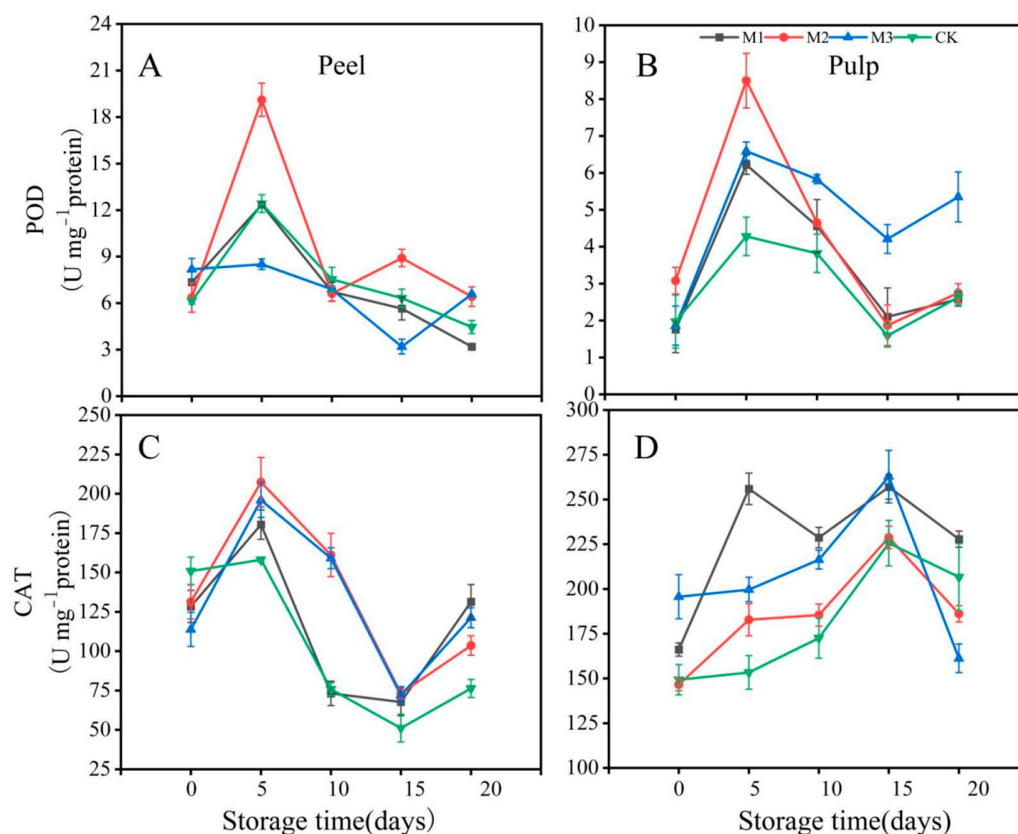


Figure 4. POD (A,B) and CAT (C,D) activities in the peel and pulp tissues of ‘Flame Seedless’ grape after MWCNT treatment. Data are means of three replicates; error bars represent SE.

The variation trend of CAT activity differed in the peel and pulp (Figure 4C,D). The CAT activity in the peel increased sharply in the first 5 days. The CAT activity of each treatment peaked on the 5th day. At this time, the CAT activity of the M2 treatment was higher than that of the control treatment and then began to decline. From the 10th day to the end of the storage period, the CAT activity of the M2 and M3 treatments was always higher than that of the control treatment (Figure 4C). On day 10, CAT activity of M2 and M3 was 2.12 and 2.09 times that of the control treatment, respectively. At the end of the storage period, the CAT activity of the M1 treatment was higher than that of the control by 41.82%. In the pulp, by contrast, the CAT activity of the M1 treatment was always above that of the control treatment throughout the storage period. The CAT activity of the M3 treatment was higher than that of the control during the first 10th day of storage, while the CAT activity of the M2 treatment was higher than that of the control treatment only on the 5th day.

The SOD activity in the grape peel was 3–15 U mg⁻¹ protein, the same as that in the pulp. The SOD activity showed an overall upward trend in the peel with the increasing storage time (Figure 5A). The SOD activity of the M1 treatment peaked on the 5th day of storage, which was higher than that of other treatments and was 1.45 times that of the control treatment. At the end of storage, the SOD activity of the M3 treatment was the highest, which was 11.06 U mg⁻¹ protein. In the pulp, the SOD activity of all treatments showed a trend of increasing first and then decreasing, and the peak SOD activity of all treatments appeared on the 10th day (Figure 5B). Among them, the SOD activity of the M1 treatment was the highest, 1.84 times that of the control treatment. At the end of storage, SOD activity of the M1, M2, and M3 treatments was 63.09%, 52.54%, and 55.43% higher than that of the control treatment, respectively.

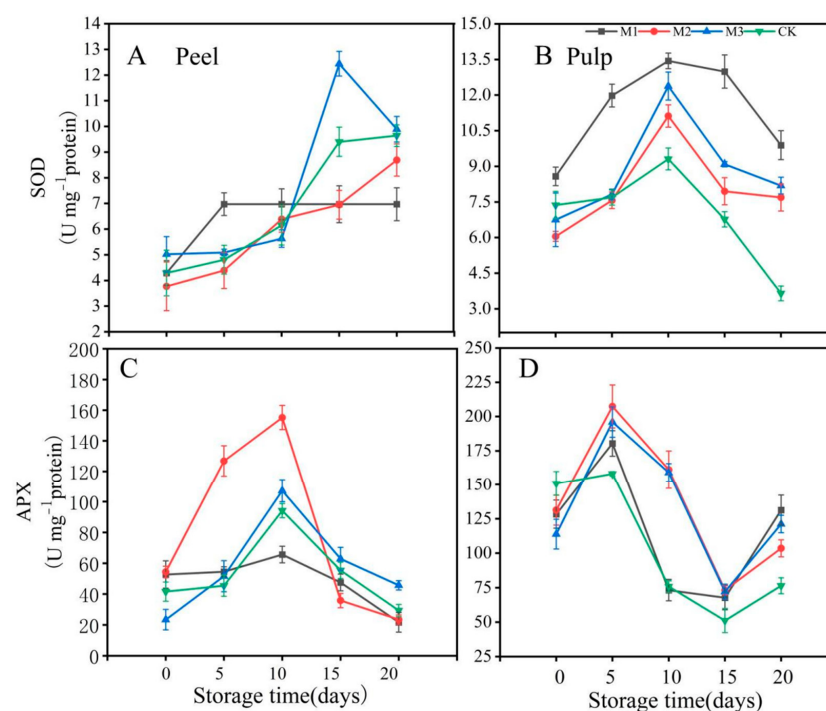


Figure 5. SOD (A,B) and APX (C,D) activities in the peel and pulp tissues of 'Flame Seedless' grape after MWCNT treatment. Data are means of three replicates; error bars represent SE.

In the peel, the APX activity of the M2 treatment was higher than that of the control in the first 10 days of storage. At its peak, it was 39.35% higher than that of the control treatment. The APX activity of the M3 treatment was higher than that of the control treatment from day 5 to the end of storage. At the end of the storage period, the APX activity of the M3 treatment was higher than that of the control treatment, which was 1.73 times that of the latter. By contrast, APX activity in the pulp peaked on day 5 for all treatments (Figure 5D). From the 5th day to the end of the storage period, APX activities of M2 and M3 treatments were higher than that of the control treatment, and at their peak, they were 17.38% and 19.11% higher than that of the control treatment, respectively.

3.4. Detection of MWCNTs in Peel and Pulp

TEM images showed the transport pathway of MWCNTs after they were ingested by plants (Figure 6). As shown in Figure 6, the MWCNTs were clearly observed in the treated sample (Figure 6C–H) while absent in the images taken from the control sample (Figure 6A,B). The results showed that MWCNTs could penetrate the epidermal cell layer and accumulate in the grape peel and pulp parenchyma cells. MWCNTs could be ingested by epidermal cells and then transported to the parenchymal cell layer.

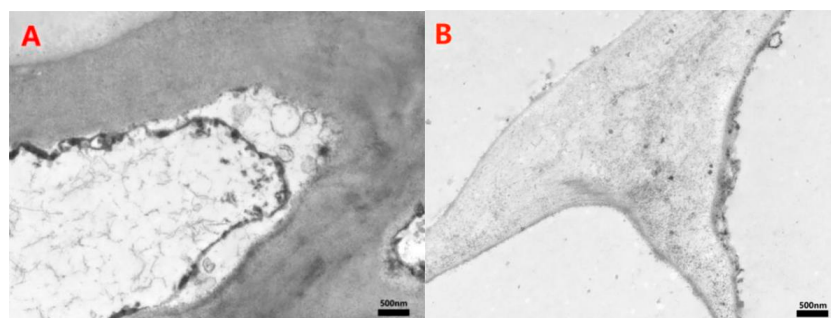


Figure 6. Cont.

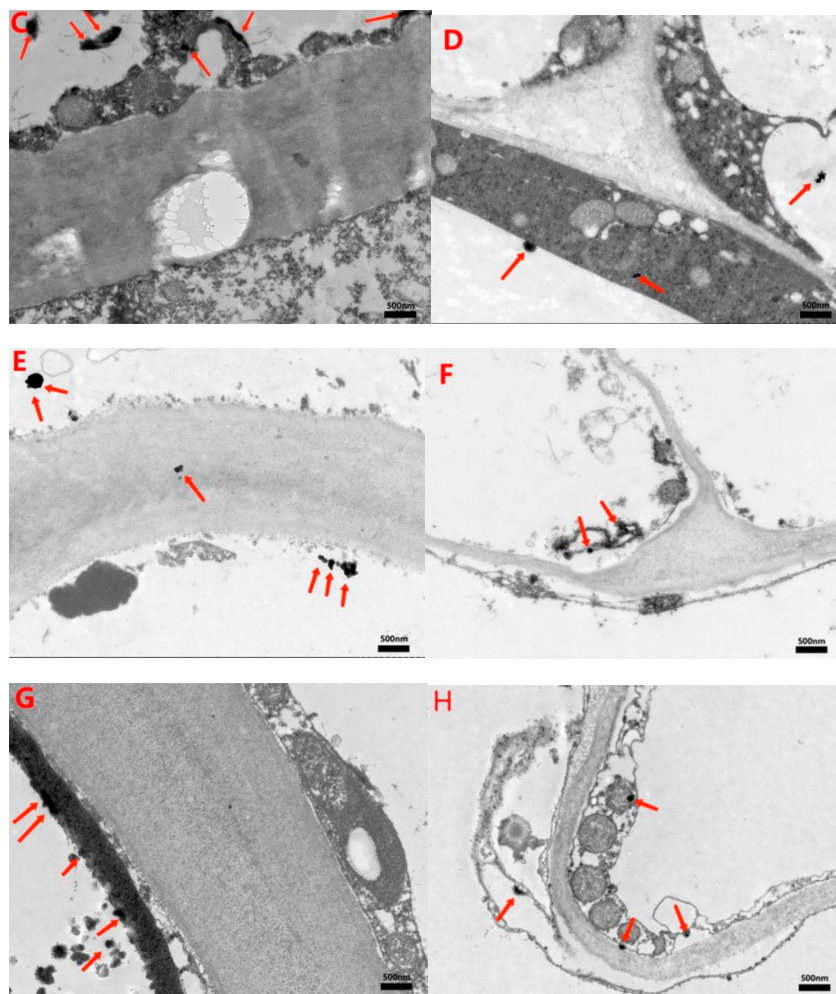


Figure 6. TEM images of the peel (C,E,G) and pulp (D,F,H) on the day of harvest after the grapes were sprayed with MWCNTs of different concentrations. MWCNTs aggregates or bundles (marked by red arrows) were detected in the intercellular spaces. (A,B) are the TEM images of peel and pulp in the control treatment, (C,E,G) are the TEM images of grape peel treated with 25, 50, and 100 mgL^{−1} MWCNTs, respectively, while (D,F,H) are the TEM images of grape pulp treated with 25, 50, and 100 mgL^{−1} MWCNTs, respectively. The scale is 500 nm.

4. Discussion

Among different types of nanomaterials, carbon nanomaterials have attracted great attention due to their unique properties and affinity for attachment, biocompatibility, and surface chemistry. They can alter physiological processes and regulate redox states in plants [31]. Most of the existing studies on MWCNTs in agricultural production focus on the whole plant system and seed germination and the interaction with callus cells [10,32,33]. However, the effects of MWCNTs on postharvest storage quality and metabolic properties of grapes are not fully understood. The results of our study showed that the application of MWCNTs significantly affected the postharvest storage quality of grapes. SSC and TA are the most important parameters for evaluating grape quality. The SSC in grapes gradually increased from day 1 to day 20 of storage. The increase in SSC might be caused by the loss of water during storage and the destruction and conversion of starch to glucose, fructose, and sucrose [34], as well as the decomposition of organic acids. Carbohydrates are the primary energy reserves. The increased carbohydrate level may promote respiratory activity, thus improving ATP levels, which in turn facilitate maintenance processes [35]. Moreover, carbohydrates may act as ROS scavengers, preserving membrane integrity [35]. By contrast, carbohydrate starvation elicits ROS formation. In this perspective, an adequate

carbohydrate level exerts a positive role, whereas an inadequate one elicits adverse effects. The pH value of the berries increases slightly during storage, which may be due to biochemical changes in the fruit, such as the decomposition of organic acids into sugars and participation in the respiratory cycle. During postharvest storage, sugars and organic acids are the primary substrates in the metabolic process [36]. AsA is a non-enzymatic antioxidant that can remove excess ROS [37]. In this study, AsA was higher in grapes treated with lower concentrations of MWCNTs than in the control group. It has been reported that low concentrations of MWCNTs can increase plant chemical activity, scavenge free radicals, and enhance antioxidant capacity [38]. Among antioxidants, ascorbate is of particular interest due to its strong potential to reduce stress events both at the cellular and organismal levels [39]. In addition, when consumed, it has profound health-promoting properties.

Oxidative damage is one of the key factors leading to the deterioration of fruit quality in the non-climacteric stage [40,41]. The accumulation of ROS accelerates the senescence of fruit, generally manifested as a high O_2^- production rate and H_2O_2 content, resulting in membrane lipid peroxidation [41,42]. Malondialdehyde (MDA) is an essential product of membrane lipid peroxidation. In this experiment, the MDA content in the pulp tissue of grapes treated with different concentrations of MWCNTs was lower than that of the control grapes (Figure 3B). During storage, more O_2^- and H_2O_2 accumulated in the peel than in the pulp (Figure 3C–F), resulting in severer membrane lipid peroxidation. However, the content of MDA in the peel and pulp was not significantly different. It is likely because the peel and pulp are different in tissue structure, antioxidant, and moisture content. These factors may account for the fact that the peel has a stronger defense against oxidative damage than the pulp. It has been reported that 50 mg L^{-1} MWCNTs cannot induce oxidative stress in *Arabidopsis* seedlings [43]. Nevertheless, high concentrations (over 100 mg L^{-1}) of MWCNTs cause oxidative damage and cell damage, which may be related to the excessive production of H_2O_2 and the increase in MDA [38]. This conclusion agrees with the report of Ghorbanpour and Hadian [10]. In this study, low concentrations of MWCNTs had stronger ROS scavenging ability in response to the induction of CAT activity in grape peel and pulp tissues, thus protecting cell membranes from lipid peroxidation. Therefore, under the action of MWCNTs at low concentrations (25 and 50 mg L^{-1}), the plant's antioxidant system can remove H_2O_2 , thereby protecting the peel and pulp cells from damage. It also partly activates the antioxidant system of grape peel and pulp during storage.

The enzymatic antioxidant system composed of antioxidant enzymes such as SOD, CAT, POD, and APX is one of the main ways to maintain the dynamic balance of ROS in fruit. SOD defends against ROS damage by converting O_2^- to H_2O_2 [41,44]. The experimental results showed that the SOD activity of M2 treatment in the peel and pulp was higher than that of other treatments and controls during the storage period. Previous studies have also obtained the same result; that is, a certain dose of MWCNTs can further improve the SOD activity of pepper seedlings. This may be attributed to the antioxidant ability of nanomaterials to mimic the actions and detoxification mechanisms of various natural enzymes [45]. In addition, it can be observed that there was no difference in catalase activity between the two tissues (Figure 4C,D). However, compared with the control treatment, MWCNTs treatment increased catalase activity, which was similar to the results of Hatami [20]. A certain dose of MWCNTs had a stronger ROS scavenging ability in zucchini seedlings in response to the induction of CAT activity, thus protecting the cell membrane from lipid peroxidation.

In this experiment, the activities of POD and APX in the grape peel and pulp of the MWCNTs treatment increased compared with the control treatment. According to Hatami [20], APX activity increased after the application of single-walled carbon nanotubes (SWCNTs), showing protection against oxidative stress and lipid peroxidation in the plasma membrane, but high doses of SWCNTs led to a decrease in APX activity. Carbon nanomaterials have cytotoxic effects on plant cells due to ROS accumulating in a dose-dependent manner [46] and overproduction [47], resulting in the structural destruction and eventual death of cells and organelles. This is consistent with a previous study

Ghosh et al. [48], which reported that only in callus cultures supplemented with 250 and 500 mg L⁻¹ MWCNTs was H₂O₂ overproduced, resulting in negative effects on plants. The complex relationship between antioxidant enzymes constitutes a ROS scavenging network in fruit. In our experiment, the average H₂O₂ content in the peel of 'Flame Seedless' grapes was several times that in the pulp. The main reason for this was that the activities of SOD and APX were lower in 'Flame Seedless' grapes. In addition, the higher content of phenol in the peel than in the pulp might be the reason for the higher activity of POD in the peel. It has been reported that there are also differences in POD activity between macaque pulp and peel [49]. In this experiment, the degree of membrane lipid peroxidation was similar when the accumulation amounts of O₂⁻ and H₂O₂ in the peel and pulp were different. This was because, under the synergistic effect, these enzymes resisted membrane lipid peroxidative damage by reducing O₂⁻ and H₂O₂ content. Previous studies have shown that the decrease or increase in enzyme activity mediated by nanomaterials may be due to the type and size of plant nanomaterials, exposure time, and experimental conditions [50]. Our experiment confirmed that, similar to other NMSs, MWCNTs were able to stimulate ROS production and lipid peroxidation in grape peel and pulp tissues. It is speculated that this was related to the activation of the antioxidant enzyme system in the two kinds of grape tissue cells induced by MWCNTs. The study by Rahmani et al. [38] showed that the antioxidant system in the leaves of *Salvia verticillata* L. was activated after spraying the leaves with low concentrations of multi-walled carbon nanotubes. Notably, after exposure to high concentrations of carbon nanotubes, oxidative stress leads to the overproduction of H₂O₂ in plant cells, which, in turn, causes tissue damage and necrosis [51].

Several studies have shown that MWCNTs can penetrate the root cells of plants, and the transport and accumulation of MWCNTs are also observed in other plant organs [19,23,52–55]. MWCNTs can also cross the plant membrane and distribute through the endosomal cycle in subcellular organelles including the nucleus, plastids, and vesicles [19,56]. The latest study by Rahmani et al. [38] showed that when a low concentration (50 mg L⁻¹) of MWCNTs is applied to the leaves of medicinal plants for a long time (15 d), MWCNTs can penetrate the epidermal cell layer and accumulate in the parenchymal cells of leaves. Therefore, the main factors affecting the uptake and transport of MWCNTs in cells were the contact time of MWCNTs with leaves, as well as the solubility and concentration of applied MWCNTs. The results of this study suggested that MWCNTs could penetrate into the pulp and peel cells after applying different concentrations of MWCNTs on the surface of grape fruit and leaves. (Figure 6). However, there is no relevant information about the mechanism of cell absorption of MWCNTs and the absorption percentage.

MWCNTs are currently being explored as a viable means of improving plant growth and productivity. In edible crops, adverse effects of nanomaterials on human health and the environment have been suggested. In this regard, no laxity in application ought to be tolerated, and disposal issues ought to be deliberated before commercial use. However, it deserves to be noted that environmental and public health concerns have also been raised regarding many chemicals currently used as preservatives in fruit and vegetables [57]. Spraying nanoparticles is an environmentally friendly and cost-effective strategy as compared to soil applications. In real-world situations, spraying the solution is undoubtedly more practical and feasible than methods targeting uptake through the stem [58]. In this case, notably, the solution volume required is considerably less than the one needed for stem uptake. This sizeable difference means spraying is not only more inexpensive for the agricultural industry, but also minimizes the environmental impact [58].

Most studies focus on the cultivation period (thus, the pre-harvest and harvest phases). Recent studies extend the current knowledge by extending the noted effects to the postharvest period [57]. Even when applied during the postharvest period, they effectively upgrade the redox state, and in this way, alleviate oxidative stress and postpone the incidence of shelf-life-terminating symptoms [57]. The present results confirmed that MWCNTs could alter ROS production and lipid peroxidation in grape pulps and peels (Figure 7). The experimental results showed that MWCNTs treatment at lower concentrations could induce

ROS and thus activate antioxidant enzymes to reduce oxidative damage in pulp and peel tissues of grapes during storage. This proved that MWCNTs at appropriate concentrations could be used as a new potential material for preserving freshness.

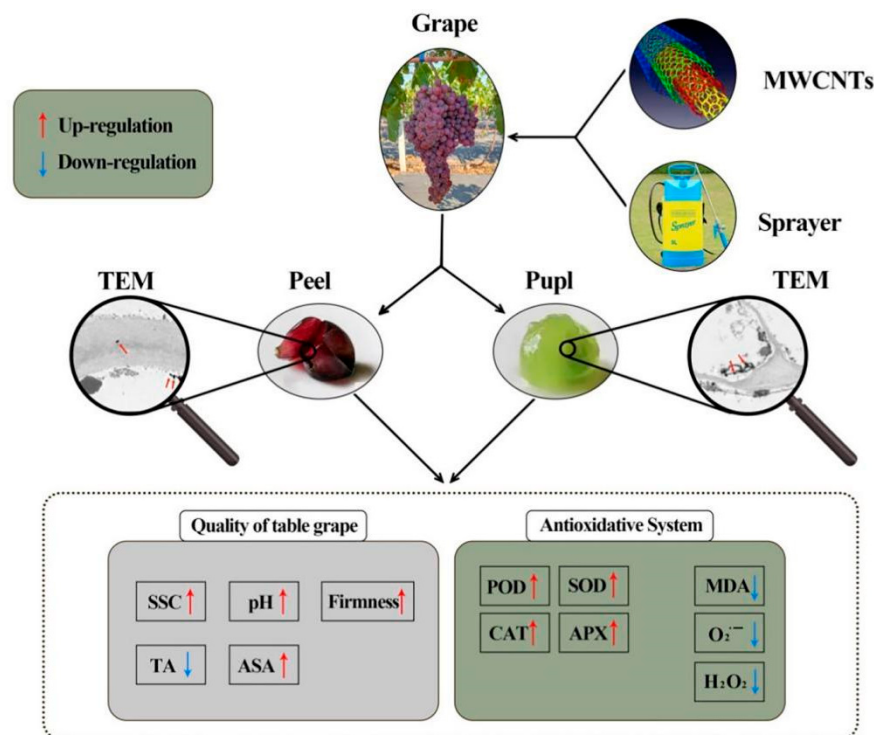


Figure 7. MWCNTs on grape quality during storage and possible mechanism of MWCNTs alleviating oxidative damage in postharvest grapes by regulating ROS metabolism.

5. Conclusions

This study showed that spraying different concentrations of MWCNTs before harvest effectively maintained the quality of grapes, thereby reducing the oxidative damage of grapes stored at 4 °C. In addition, lower concentrations of (25 and 50 mg L⁻¹) MWCNTs treatment increased the activity of antioxidant enzymes and reduced the excessive accumulation of ROS in grape peel and pulp, which contributed to reducing the oxidative damage of ‘Flame Seedless’ grapes during storage. These results indicate that MWCNTs at a specific concentration can be used as a new biosynthetic antioxidant inducer for grape preservation. However, TEM images showed that MWCNTs would penetrate into the peel and pulp tissues. Thus, although MWCNTs are beneficial to grape preservation, for food safety assessment, the internalization and distribution mechanism of MWCNTs in plant cells and their impact on human health should be further studied.

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