



## Article

## Effects of 1-MCP Treatment on Postharvest Fruit of Five Pomegranate Varieties during Low-Temperature Storage

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**Abstract:** Pomegranate fruit production and consumption are restricted by appropriate postharvest handling practices. 1-MCP (1-methylcyclopropene) is a natural preservative of fruits and vegetables; however, its effects on the storage of different pomegranate varieties have not been extensively investigated. Herein, the effects of 1.0  $\mu\text{L L}^{-1}$  1-MCP on postharvest pomegranate fruit of three soft-seed ‘Mollar’, ‘Malisi’, and ‘Tunisan soft seed’ and two semi-soft-seed ‘Moyuruanzi’ and ‘Dongyan’ were investigated over 90 d (days) under low-temperature storage at  $4 \pm 0.5$  °C with a relative humidity of 85–90%. Several indexes of exterior and interior quality were recorded, the sensory quality was evaluated, and the respiration and ethylene production were also determined. The results showed that peel browning was generally more severe in the soft-seed varieties than in the semi-soft-seed varieties. Significantly lighter peel browning presented in the three soft-seed fruits from 45 d after the 1-MCP treatment, with 35%, 19%, and 28% less than those controls at 90 d, correspondingly. However, 1-MCP only significantly decreased peel browning in the semi soft-seed fruits at 60 days. A prominent decrease in weight loss was recorded in all five varieties, with ‘Malisi’ showing the largest and ‘Dongyan’ the smallest difference between the 1-MCP and control treatments. Through the results of color, physiological, and chemical changes, as well as sensory properties, better color and total acceptance were found with higher titratable acids and vitamin C but with decreased anthocyanins in most fruits treated with 1-MCP. In contrast to the control, remarkable suppression of ethylene production peaks in all whole fruits and periodical increase in respiration rates in the soft-seed whole fruits were activated at 30–60 d after storage by the 1-MCP treatment, roughly when peel browning occurred and began increasing. Overall, our findings provided a crucial foundation for extending the application of 1-MCP in postharvest preservation of pomegranates.

**Keywords:** postharvest pomegranate; 1-MCP; peel browning; fruit quality; weight loss; respiration; ethylene production



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## 1. Introduction

Pomegranates are native to the Middle East and widely cultivated worldwide [1]. Pomegranates (*Punica granatum* L.) belonging to *Punica* of *Lythraceae* are well-liked for their distinctive flavors and biologically active ingredients [2,3]. In China, the pomegranate harvest season typically lasts from August to October. Different protocols for large storing of pomegranates have been developed in different countries [4–6]. However, they are still highly perishable commodities along the postharvest value chain, from harvest to consumption, because of peel browning, weight loss, color and flavor deterioration, chilling

injury, and quality loss, which reduce the storability and affect the consumer acceptance of the fruits, leading to direct financial loss [5–7].

Low-temperature storage is widely and effectively used for the postharvest preservation of pomegranates according to Liu et al. (cv. soft-seed ‘Tunisian’ and hard-seed ‘Jingpitian’ and ‘Lishanhong’) [8], Belay et al. and Lufu et al. (cv. hard-seed ‘Wonderful’) [7,9], Shi et al. (cv. soft-seed ‘Tunisian’ and hard-seed ‘Yudazi’) [10], and Caleb et al. (cv. ‘Acco’ and ‘Herskowitz’) [4]. Pomegranate fruit can be categorized into three categories based on aril hardness, namely, soft-seed, semi-soft-seed, and hard-seed types. Pomegranate fruits with hard seeds, such as ‘Yudazi’, have a longer storage life of 90–100 days at room temperature. The ‘Tunisian soft seed’, ‘Mollar’, and ‘Malisi’ varieties of soft-seed pomegranates, which were recently imported from Tunisia and Israel into China [11–14], have a storage life of only 30 days at room temperature. Little is known regarding the postharvest storage of the soft-seed varieties mentioned above and the Chinese native varieties, semi-soft-seed ‘Moyuruanzi’ and ‘Dongyan’. Studying the quality variations of the five varieties during low-temperature storage was one of the aims of this study.

1-Methylcyclopropen (1-MCP) is a highly effective, non-toxic, and chemically stable preservative widely used to extend postharvest storage time and prevent the decay of horticultural products [15]. Studies have shown that low temperature combined with 1-MCP treatment can effectively delay the postharvest ripening and senescence process of most climacteric fruits, such as bananas, apples, and peaches, with the effects of promoting freshness and extending shelf-life [15–18]. However, 1-MCP treatment also positively affects postharvest storage and preservation of non-climacteric fruits [19–23]. The treatment of  $1 \text{ mL L}^{-1}$  1-MCP effectively delayed the pedicel browning of fresh fruits of different grape cultivars [19]. With the ability to inhibit the increase in the respiration intensity of winter jujubes during low-temperature storage, 1-MCP treatment can ensure excellent quality by maintaining the preferable pericarp color and reducing weight loss and moldy rate [20]. The treatment of  $300 \text{ nL L}^{-1}$  1-MCP can significantly decrease the lychee peel browning of ‘Mauritius’ and ‘McLean’s Red’ under MAP packaging, while maintaining the vibrant color of the peel [21].

The non-climacteric nature of pomegranate fruit during development and ripening has been proven [6]. Gamrasni et al. [24] found that the pomegranate ‘Wonderful’ fruit maintained a good flavor compared to the control after the  $900 \text{ nL L}^{-1}$  1-MCP treatment and storage at  $7^\circ\text{C}$  for 120 days. Other researchers found that  $1.0 \text{ }\mu\text{L L}^{-1}$  1-MCP effectively promoted ‘Tunisia’, ‘Wonderful’, ‘Tunisian soft-seed’, and ‘Dahongpao’ pomegranate fruit quality by reducing peel browning rates and preserving flavors during low-temperature storage [25–28]. Although non-climacteric fruit exhibit a declined low respiration and low ethylene production during maturation and ripening, ethylene does participate in the regulation of maturation and some physiological changes [29]. According to Valdenegro et al. [5], 1-MCP as a typical ethylene antagonist did not significantly inhibit the respiration rate and ethylene release of ‘Wonderful’ during low-temperature storage at  $2^\circ\text{C}$ . Differently, other researchers observed  $1.0 \text{ }\mu\text{L L}^{-1}$  1-MCP significantly reduced the respiration intensity of ‘Tunisia’ and ‘Tunisian soft-seed’ during low-temperature storage at  $4^\circ\text{C}$  [25–27]. It has been found that the effects of a 1-MCP application on respiration, ethylene production, and fruit quality may depend on the variety, tissue, and storage conditions [5,23,30]. 1-MCP is a natural preservative of fruits and vegetables; however, its effects on the storage of different pomegranate varieties have not been investigated extensively. Therefore, this work also aimed to explore the effect of the 1-MCP treatment on postharvest fruit of the five different pomegranate varieties mentioned above during a low-temperature storage. Our work will contribute to the practical application of 1-MCP in postharvest preservation of pomegranates.

## 2. Materials and Methods

### 2.1. Plant Materials

Pomegranate fruit of soft-seed varieties, ‘Tunisian soft seed’, ‘Malisi’, and ‘Mollar’, as well as semi-soft-seed varieties, ‘Moyuruanzi’ and ‘Dongyan’, from a 8-year-old commercial vineyard (Liugou Village, Gaocun Town, Xingyang City, Henan, China) under natural field conditions were harvested at commercial maturity by experienced staff (typical peel and aril color and juice soluble solids higher than  $>14\%$ ). At harvest, the undamaged and healthy fruits were representatively selected for uniformity in color, size (about 0.3–0.4 kg/fruit), and appearance without sunburn, cracks, bruises, cuts, decay, or disease. The harvest time of ‘Tunisian soft seed’, ‘Malisi’, ‘Mollar’, and ‘Moyuruanzi’ were on 5 October, and it was on 27 October 2021 for ‘Dongyan’, a late-mature landrace. Three-hundred pomegranate fruit of each variety were transported into the laboratory within 3 h after harvest and then were sorted, cleaned, and arranged into three replications.

### 2.2. 1-MCP Treatment

A total of ninety harvested pomegranate fruit of each variety for one replication were randomly and equally put in fifteen flat polyethylene bags with the specification of 1200 mm long, 800 mm wide, and 0.05 mm thick. They were either left untreated or treated with AnsiP-S stickers (1-methylcyclopropene, Lytone Enterprise, Inc., Taipei, Taiwan, China) and then sealed for 24 h at  $4 \pm 0.5$  °C with a relative humidity of 85–90%. The treatment method of Guo et al., 2019 [27] was used, and the effective concentration of 1-MCP was  $1.0 \mu\text{L L}^{-1}$ . This concentration of 1-MCP has been verified with good effect on preservation of several different pomegranate varieties such as ‘Tunisian’, ‘Wonderful’, and ‘Dahongpao’ [25–28]. Thereafter, all bags were converted to a semi-closed state and transferred to the low-temperature storage at  $4 \pm 0.5$  °C for up to 90 days (d). Six pomegranate fruit for each treatment for one replication were taken out from the low-temperature storage for data analysis at 0, 15, 30, 45, 60, and 90 d after harvest. Fruit quality, ethylene production, and respiration rate were assessed in whole fruit and arils. Arils were artificially and quickly separated and transferred to measurement or making juices. Decreasing the effects of the higher room temperature than arils themselves from the low-temperature surroundings should be considered as much as possible. Aril juices were prepared by squeezing the arils through a double-layer gauze and then were frozen at  $-20$  °C for further analysis. The analyses were performed for all samples at the same time.

### 2.3. Exterior Quality Index

According to Kashash et al. [30], pomegranate peel browning, the principal non-pathogenic disorder occurring on the fruit surface, was divided into five levels based on the browning areas: level 0 (no browning symptoms and 0 browning area), level 1 (browning areas between 1 and 25%), level 2 (browning areas between 26 and 50%), level 3 (browning areas between 51 and 75%), and level 4 (browning areas  $> 75\%$ ). Then, the browning index was calculated according to the formula described by Zhang et al. [28]: the browning index =  $\sum (\text{browning level} \times \text{the number of fruits of that level}) / (5 \times \text{total number of fruits})$ . A higher browning index represented peel browning more severe. From all arils of each sample, 100 arils were randomly selected and weighted to determine the hundred-aril mass with values being presented as g. The  $L^*$ ,  $a^*$ , and  $b^*$  values of aril color were measured using a HP-C210 visible light colorimeter (Hanpu Photoelectric Technology Co., Ltd., Shanghai, China) to obtain the brightness values ( $L^*$ ), the chromatic values [ $C^* = (a^{*2} + b^{*2})^{1/2}$ ], and the hue angle values [ $H^* = \tan^{-1}(b^*/a^*)$ ].

### 2.4. Interior Quality Index

The total soluble solids of aril juice were measured by a WTY handheld refractometer (Chengdu Qingyang Huarui Optical Instrument Factory, Chengdu, China), with values being presented as Brix degrees (%). The titratable acid contents were tested using the acid–base neutralization titration method by titrating 5 mL of juice to reach the endpoint

of pH 8.2 with 0.1 M NaOH and recording the titration volume. The resulting data were expressed as citric acid percentage. The ratios of soluble solid and titratable acid were then calculated. The methods described above were all based on Gao et al., 2022 [31], and each measurement was repeated at least three times.

The vitamin C contents of aril juice were detected by a 2,6-dichlorophenol indophenol (DCIP) titration method described by Gao et al., 2022 [31]. About 0.5 g of fresh arils, ground with liquid nitrogen, was mixed with 50 mL of 2% (*m/v*) oxalic acid solution. Then, 10 mL of the solution was transferred to a triangular flask (50 mL), and the DCIP solution that had been calibrated was immediately performed for sample solution titration. The terminal point was recorded with a reddish appearance at 15 s fadeless. Vitamin C contents of each sample were determined by the consumed volume of the DCIP solution. Total anthocyanin contents were determined according to the pH differential method described by Shi et al., 2022 [32]. Absorbance was measured in a UV-VIS spectrophotometer (L9, INESA, Inc., Shanghai, China) at 510 and 700 nm in buffers at pH 1.0 and 4.5, respectively. The results were expressed as milligrams of cyanidin-3-glucoside per L of pomegranate juice. Each detection above was performed three times.

### 2.5. Ethylene Production

After the postharvest treatments (control, 1-MCP), ethylene production of whole fruit and arils were analyzed at different periods after 0, 15, 30, 45, 60, and 90 days during storage using gas chromatography (GC; GC-2010 PLUS, Shimadzu, Kyoto, Japan) with a flame ionization detector [26]. Two whole fruits were randomly selected from each sample and placed into a 2 L plastic box to rest for 3 h at room temperature. The plastic boxes were cubic and had a sealed lid fitted with a rubber stopper. Then, 1000  $\mu\text{L}$  of gas was taken with a micro-sampler from the cubic plastic box and injected into a GC Packed Column (GDX-502, Shimadzu, Kyoto, Japan) for analysis. Nitrogen was used as the carrier gas at the flow rate of  $40 \text{ mL min}^{-1}$ , with the injection port set to  $100^\circ\text{C}$  and the detector (FID, Lanzhou Institute of Chemical Physics, Lanzhou, China) to  $150^\circ\text{C}$ . A  $10 \mu\text{L L}^{-1}$  ethylene standard was used for equipment calibration. Ethylene production [ $\mu\text{L h}^{-1} \text{ kg}^{-1}$  (FW)] =  $c \times V \times m^{-1} \times t^{-1}$ , where *c* is the ethylene content as determined by gas chromatography; *V* is the sealed container's space volume—sample volume (L); *m* is the sample mass (kg); *t* is the standing time (h). The arils of each sample were weighted 0.2–0.3 kg to analyze the respective ethylene production according to this method. Each experiment was repeated at least three times.

### 2.6. Respiration Rate

Two whole fruit from each sample were taken out from low temperature and kept still for 3 h at room temperature before being placed into a 2 L cubic plastic box with a sealed lid fitted with a rubber stopper for another 3 h at room temperature. Finally, carbon dioxide concentration was measured using the portable carbon dioxide analyzer (F-950, FELIX Company, Camas, WA, USA), and respiration rates were calculated according to Guo et al. [27]. Respiration rate [ $\text{mg h}^{-1} \text{ kg}^{-1}$  (FW)] =  $c \times V \times 44 \times 273 \times (m \times t \times 22.4 \times 293)^{-1}$ , where *c* is the carbon dioxide concentration; *V* is the sealed container's space volume—sample volume (L); *m* is the sample mass (kg); *t* is the storage time (h); 44: the molar mass value of carbon dioxide, 44 kg/mol; 273: the thermodynamic temperature at  $0^\circ\text{C}$  in standard condition, 273 k; 22.4: the volume of 1 mmol gas in standard condition, 22.4 mL/mmol; 293: the thermodynamic temperature at  $20^\circ\text{C}$  in standard condition, 293 k. According to the method mentioned above, the arils of each sample were weighted 0.2–0.3 kg to analyze the corresponding respiration rates. Each experiment was repeated at least three times.

### 2.7. Sensory Evaluation

After 90 days of low-temperature storage, six fruit from each sample were randomly selected. Descriptive sensory analyses were performed by 10 panelists, developed by Osondu et al. [33], which were then thoroughly evaluated through four aspects. First, fruit



pericarp and aril were scored according to several color grades: those with superior color received 10–9 points; those with good color received 8–7 points; those with acceptable but limited marketability received 6–5 points; and the rest received under 5 points. Second, fruit were scored according to their level of flavor: if they had a typical flavor, they received 9–10 points, if good, they received 7–8 points; if moderately acceptable, they received 6–5 points, if acceptably but limitedly marketable, they received 3–4 points, and the rest received 1–3 points. Third, fruit were scored according to the level of odor grades: fruit with no odor received 9–10 points; fruit with a little odor was given 7–8 points; fruit with some odor received 6–5 points; if obvious but tolerable odor, they received 3–4 points, and the rest received 1–3 points. Finally, overall acceptance would be directly scored through comprehensive sense: fruit with 10–9 points were liked; fruit with 8–7 points were moderately liked; fruit with 6–5 points were not liked nor disliked; fruit with 4–3 points were moderately disliked; and fruit with 2–1 points were especially disliked. The final score used to assess the sensory qualities of each sample was the average value of the aspects mentioned above.

## 2.8. Statistical Analysis

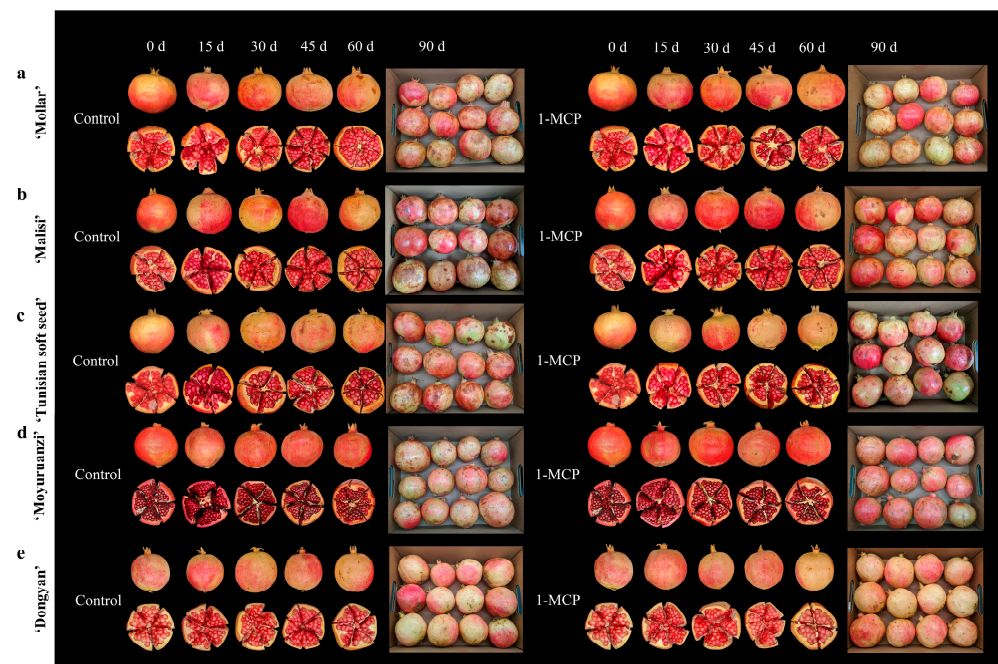
One-way analysis of variance was used to analyze all the data using Statistical Package for Social Sciences version (IBM. SPSS Inc., Chicago, IL, USA), with three replications of each experiment. Duncan's multiple comparison was applied at a  $p = 0.05$  probability level to evaluate significant differences among data points and between the control and 1-MCP treatments. GraphPad Prism 9 (GraphPad Software Inc., San Diego, CA, USA) was used to draw the figures, and Adobe Photoshop CC 2017 (Adobe Systems Inc., San Jose, CA, USA) was used to combine them.

## 3. Results

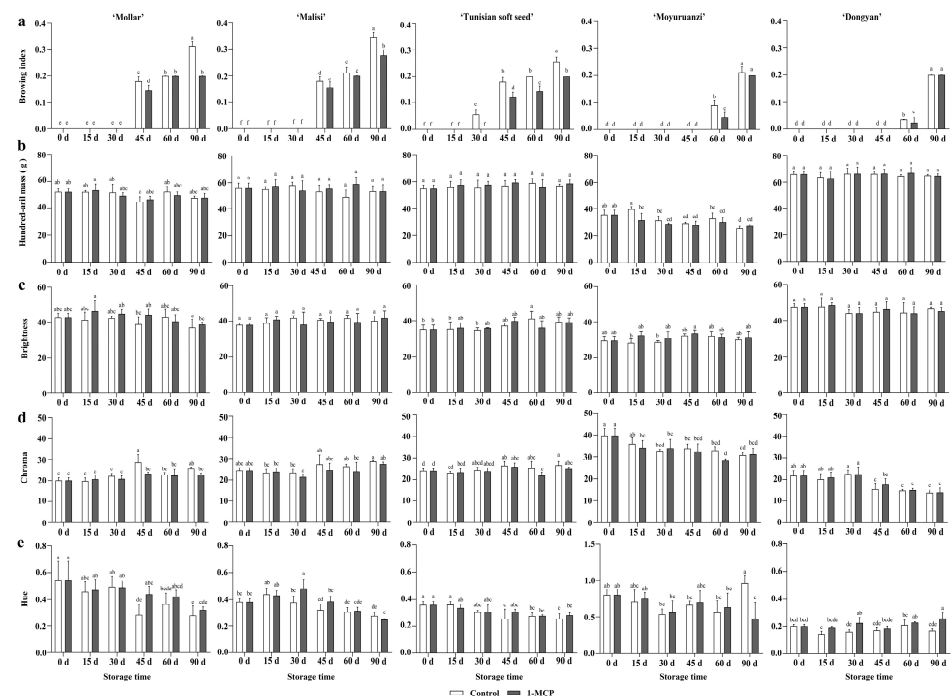
### 3.1. Effects of 1-MCP on Exterior Quality

#### 3.1.1. Browning Index

The investigated five pomegranate varieties except 'Dongyan' experienced obviously fewer browning lesions in fruit treated with 1-MCP compared to the untreated control, especially 90 d after low-temperature storage. When 'Dongyan' and 'Moyuruanzi' fruit were treated with 1-MCP, there were fewer browning lesions than in fruit from other types or the control (Figure 1). The Browning index of the five varieties increased with storage time. Rather, the browning in the peels of the treated or untreated soft-seed fruit of 'Mollar' and 'Malisi' occurred and then rapidly developed from 45 d; before that, there was no visible browning. In 'Tunisian soft seed', peel browning occurred early at 30 d with the control, when none was found with the 1-MCP treatment. The beginning time point of peel browning in the two semi-soft-seed fruit was at 60 d (Figure 2a). With the 1-MCP treatment, 'Mollar', 'Malisi', and 'Tunisian soft seed' reduced the peel browning index from 0 at harvest to 0.20, 0.28, and 0.20 after 90 days of storage, which were significantly 35%, 19%, and 28% less than those controls (0.31, 0.34, and 0.28), respectively (Figure 2a). On the contrary, peel browning presented from 60 d after storage both in 'Moyuruanzi' and 'Dongyan' semi-soft-seed fruit. At this point, their peel browning indexes with the 1-MCP treatment (0.044 and 0.022, respectively) were significantly lower than those with the control (0.089 and 0.033, respectively). However, they exhibited no significant difference between the control and 1-MCP treatments at 90 days (around 0.20) (Figure 2a). Additionally, the results showed that compared to semi-soft-seed fruit, these soft-seed fruits had a considerably greater peel browning (Figures 1 and 2).



**Figure 1.** 1-MCP treatment affected the macro performance of the five pomegranate varieties during the low-temperature storage. (a) 'Mollar'; (b) 'Malisi'; (c) 'Tunisian soft seed'; (d) 'Moyuruanzi'; (e) 'Dongyan'. Control: untreated control; 1-MCP: samples that were treated with 1-MCP stickers. All the fruit were stored at the low temperature of 4 °C and with the relative humidity of 85–90%.



**Figure 2.** 1-MCP treatment affected the exterior quality index of the five pomegranate varieties during low-temperature storage, which included browning index (a), hundred-aril mass (b), brightness values (c), hue values (d), and chroma values (e). Control: untreated control; 1-MCP: samples that were treated with 1-MCP stickers. All the fruit were stored at the low temperature of 4 °C with the relative humidity of 85–90%. Data represent mean values of the replications, and the error bars represent standard deviations of the means. Different letters indicate significance differences at a significant level of  $p = 0.05$  using Duncan's test.

### 3.1.2. Color

As shown in Figure 2b, the 1-MCP treatment did not affect the hundred-aril mass of these five varieties during low-temperature storage, with ‘Moyuruanzi’ being the lowest. The 1-MCP treatment also caused no significant change in aril brightness of these five varieties compared to the untreated control, which maintained a stable level during storage (Figure 2c). However, their chromatic values showed an increase in control arils of three soft-seed varieties but a decrease in two semi-soft-seed varieties during storage. Nevertheless, these values showed no variation after the 1-MCP treatment (Figure 2d). Hue values decreased significantly from harvest with a slight increase at 60–90 d after storage in control soft-seed arils. With the treatment of 1-MCP, hue values were obviously higher at 30–45 d compared to the control. These results indicated that 1-MCP could contribute to maintaining the red color of these pomegranate arils. Additionally, the red color of ‘Moyuruanzi’ arils was stronger and more saturated for their higher chromatic and hue values than other varieties (Figure 2e).

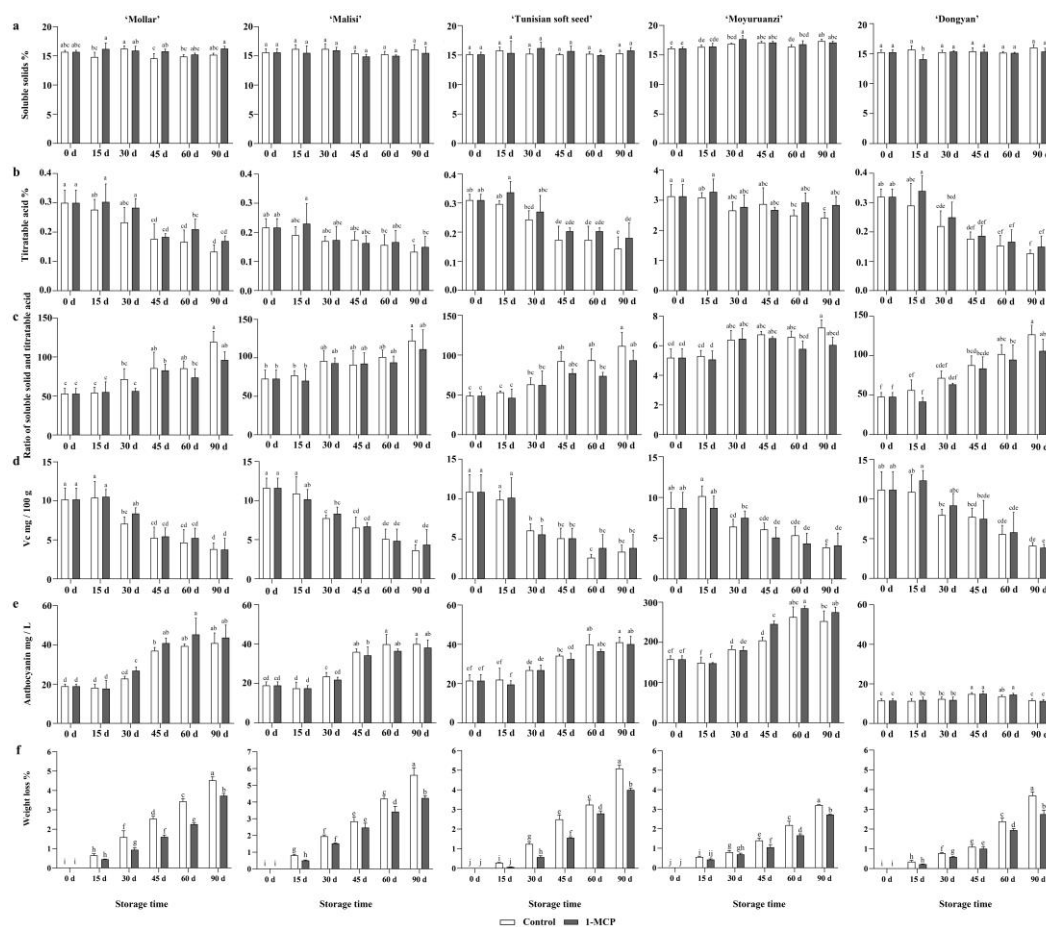
### 3.2. Effects of 1-MCP on Interior Quality

#### 3.2.1. Total Soluble Solids and Titratable Acids

Figure 3a,b depicts the variations in total soluble solids and titratable acids of the five pomegranate varieties. Total soluble solids in control arils of all five varieties essentially did not change much during storage; on the contrary, titratable acids showed a substantial downward trend, particularly after 30 d. Total soluble solids and titratable acids of ‘Moyuruanzi’ retained 16.1–17.7% and 2.41–3.28%, respectively, which were both higher than those of the other varieties. The titratable acids of ‘Malisi’ were the lowest, ranging between 0.13% and 0.21%. Ratios of total soluble solids and titratable acids in these arils, except ‘Moyuruanzi’, dramatically rose throughout storage, rising from around 50 at 0 days to about 100 at 90 days. In ‘Moyuruanzi’ arils, the ratios exhibited a significant increase from 5.2 to 6.1, which might be the result from its attribute of extreme high titratable acids (Figure 3c). With 1-MCP treatment, total soluble solids were unaffected at almost all storage stages; however, the decrease in titratable acids were apparently restrained (Figure 3a,b). At 90 d after storage, the titratable acids were 27.5%, 12.5%, 25.6%, 18.0%, and 18.4% greater in arils of the five varieties with the treatment of 1-MCP than those in controls in the order depicted in Figure 3b. Associated with the slow decrease in titratable acids in 1-MCP-treated arils of the five varieties with storage duration, their ratios of total soluble solid and titratable acid were lower than those in control arils (Figure 3c).

#### 3.2.2. Vc and Anthocyanins

Figure 3d also presented a serious and significant loss of Vc content in arils of the five pomegranate varieties with storage duration. At 0 d, their Vc in control arils ranged from 87 to 116 mg kg<sup>-1</sup>, but they had decreased to 36–41 mg kg<sup>-1</sup> at 90 d. Although a slight increase in Vc in arils with 1-MCP treatment were observed at 30 d except for ‘Tunisian soft seed’ at 60 d, 1-MCP was unable to reverse these decreases finally. Anthocyanins are important phenolic compounds presented in pomegranates that also affect the color of fruits [34]. To verify the effect of 1-MCP on postharvest pomegranate fruit during low-temperature storage, anthocyanin contents in arils were investigated in the current study (Figure 3e). In contrast with declining Vc content, the anthocyanin content in arils displayed a significant increase with the storage period. Higher anthocyanins ranging from 148.2 to 284.8 mg L<sup>-1</sup> were found in ‘Moyuruanzi’ arils in comparison with those in other arils. Three soft-seed arils had 17.6–43.7 mg L<sup>-1</sup> anthocyanins, and ‘Dongyan’ had the lowest 11.4–15.0 mg L<sup>-1</sup>. Pomegranate arils of ‘Mollar’, ‘Moyuruanzi’, and ‘Dongyan’ treated with 1-MCP showed a slight increasing anthocyanins after 30 d during storage.



**Figure 3.** 1-MCP treatment affected the interior quality index of the five pomegranate varieties during low-temperature storage, which included total soluble solid contents (a), titratable acid contents (b), ratios of soluble solid and titratable acid (c), Vc contents (d), anthocyanins (e), and weight loss (f). Control: untreated control; 1-MCP: samples that were treated with 1-MCP stickers. All the fruit were stored at the low temperature of 4 °C with the relative humidity of 85–90%. Data represent mean values of the replications, and the error bars represent standard deviations of the means. Different letters indicate significant differences at a significance level of  $p = 0.05$  using Duncan's test.

### 3.2.3. Weight Loss

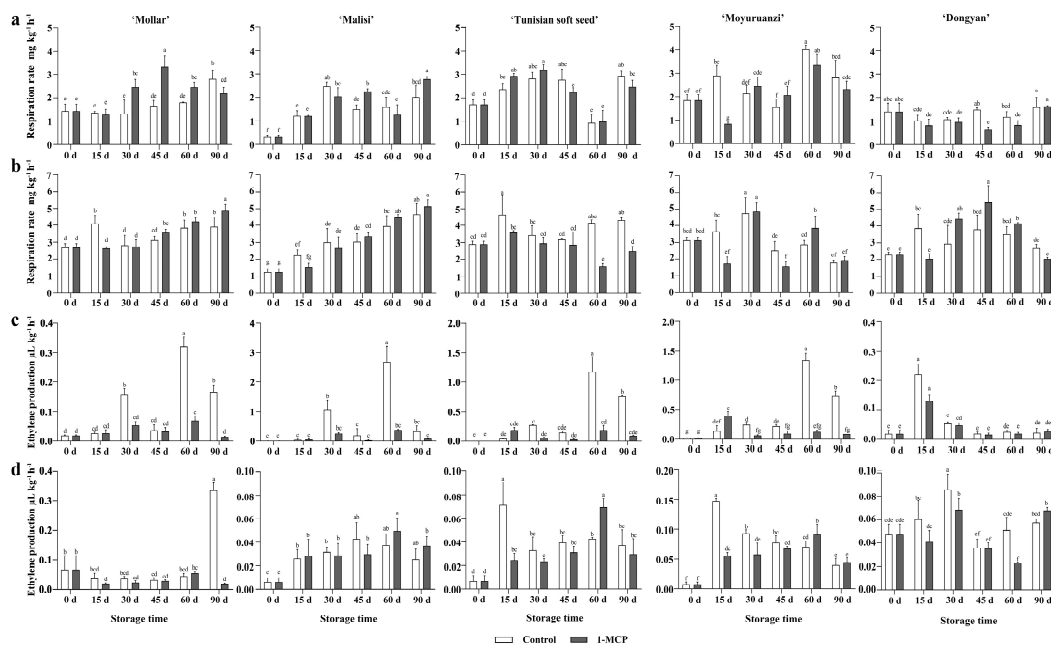
On average, increasing storage time from 0 d to 90 d increased weight loss across all varieties, both of the 1-MCP and control treatments. After the 90 d storage period, the highest (5.33%) and lowest (2.75%) weight losses were recorded in the fruit of 'Malisi' with the control and 'Moyuruanzi' with the 1-MCP treatment, respectively (Figure 3f). However, weight loss was significantly lesser for all fruit treated with 1-MCP, compared to the untreated control. Among the five varieties, the greatest difference of increasing weight loss between the 1-MCP treatment and the control was recorded in 'Malisi' fruits, followed by 'Mollar', and the smallest was in 'Dongyan' (Figure 3f). These analyses demonstrated that 1-MCP was an effective treatment for weight loss.

## 3.3. Effects of 1-MCP on Respiration Rate and Ethylene Production

### 3.3.1. Respiration Rates of Whole Fruit and Arils

In most cases, the respiration of whole fruit and arils of the five varieties both with the 1-MCP and control treatments displayed increases with fluctuations during storage at the low temperature (Figure 4a,b). Among whole fruit varieties, the lowest respiration rates were recorded in 'Dongyan' with no more than  $1.6 \text{ mg kg}^{-1} \text{ h}^{-1}$  (Figure 4a). Compared with the whole fruit of the control, respiration rates were significantly increased by the

1-MCP treatment in soft-seed ‘Mollar’, ‘Malisi’, and ‘Tunisian soft seed’ at 30–45 d after storage, but they were mainly declined in semi-soft-seed ‘Moyuruanzi’ and ‘Dongyan’ (Figure 4a). Nevertheless, at 90 d, the increment percentages of respiration rates from 0 d in whole fruit of ‘Mollar’, ‘Malisi’, ‘Tunisian soft seed’, and ‘Moyuruanzi’ (52.3%, 52.6%, 43.3%, and 25.8%, respectively) were recorded as 42.5%, 56.5%, 25.9%, and 26.2% lower, respectively, in fruits with the 1-MCP treatment than those with the control (94.9%, 109.1%, 69.1%, and 96.3%, respectively). For ‘Dongyan’, it (14.0%) was 2% higher (16.0%) (Figure 4a). These results indicated that 1-MCP increased whole fruit respiration in the soft-seed varieties during the middle storage. Still, it prevented a subsequent increase in respiration, including the semi-soft-seed ‘Moyuruanzi’.



**Figure 4.** 1-MCP treatment affected respiration rate and ethylene production of the five pomegranate varieties during low-temperature storage. (a) Respiration rates of whole fruit; (b) respiration rates of arils; (c) ethylene production of whole fruit; (d) ethylene production of arils. Control: untreated control; 1-MCP: samples that were treated with 1-MCP stickers. All the fruit were stored at the low temperature of 4 °C with a relative humidity of 85–90%. Data represent mean values of the replications, and the error bars represent standard deviations of the means. Different letters indicate significant differences at a significance level of  $p = 0.05$  using Duncan’s test.

Among arils of varieties, the respiration rates of ‘Mollar’ and ‘Malisi’ both with the 1-MCP and control treatments increased progressively from around 2.0 mg kg<sup>-1</sup> h<sup>-1</sup> to approximately 4.0 mg kg<sup>-1</sup> h<sup>-1</sup>. However, a significant decrease of 34.6% was observed in ‘Mollar’ arils caused by the 1-MCP treatment at 15 d after storage. Simultaneously, similar decreases of 10%, 24.1%, 52.9%, and 47.2% were observed in ‘Malisi’, ‘Tunisian soft seed’, ‘Moyuruanzi’, and ‘Dongyan’, respectively (Figure 4b). After that, the respiration rate of ‘Tunisian soft seed’ arils with 1-MCP treatment was invariably lower than that with control. Although respiration rates of ‘Moyuruanzi’ and ‘Dongyan’ exhibited different increases after 15 d, the increment percentages of their respiration rates from 0 d to 90 d after the 1-MCP treatment were 4.5% and 29.5% lower than those after the control treatment, respectively (Figure 4b). Through the analysis above, it was suggested that 1-MCP effectively declined aril respiration rates in all five varieties at the beginning of storage and hindered the rates of aril respiration in ‘Tunisian soft seed’, ‘Moyuruanzi’, and ‘Dongyan’ at the end.



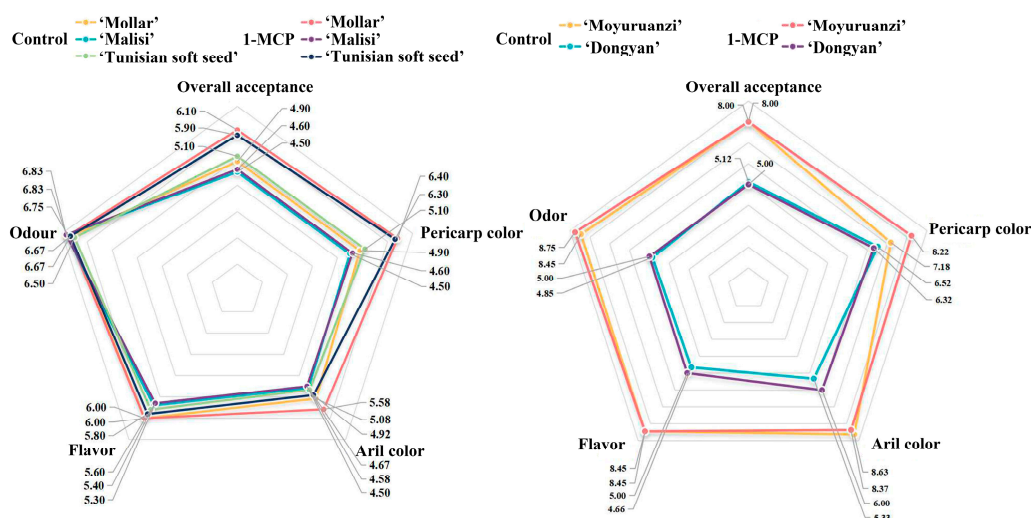
### 3.3.2. Ethylene Production of Whole Fruit and Arils

The analysis of variance among the experimental effects showed that 1-MCP significantly affected the ethylene production in the treated whole fruit and arils of the five pomegranates, which are referred to as a kind of non-climacteric fruit (Figure 4c). The changed pattern was quite different from that of the respiration rates, especially in whole fruit. The 1-MCP treatment significantly suppressed ethylene production peaks of whole fruit of different varieties, mainly at 30 d, 60 d, and 90 d, except for 'Dongyan', which peaked only at 15 d. At another period, the ethylene production of whole fruit of the control remained at  $0.04 \mu\text{L kg}^{-1} \text{h}^{-1}$  and  $0.76 \mu\text{L kg}^{-1} \text{h}^{-1}$  during storage; by contrast, it was  $0.083 \mu\text{L kg}^{-1} \text{h}^{-1}$  at most after the 1-MCP treatment. Among the whole fruit of five cultivars, the highest ethylene production of  $2.67 \mu\text{L kg}^{-1} \text{h}^{-1}$  at 60 d was recorded in 'Malisi' of the control that declined into  $0.38 \mu\text{L kg}^{-1} \text{h}^{-1}$  by 1-MCP at 90 d (Figure 4c).

Among arils of varieties both of the 1-MCP and control, ethylene production was always under  $0.094 \mu\text{L kg}^{-1} \text{h}^{-1}$  during storage, except with a quite high value of  $0.34 \mu\text{L kg}^{-1} \text{h}^{-1}$  in 'Mollar' at 90 d (Figure 4d). Despite the fact that ethylene production displayed quite lower levels in arils than those in whole fruit, some effects were also found in arils by 1-MCP. Compared to the control, the ethylene productions were slowed down in arils of all five varieties of the 1-MCP treatment at 15–45 d after storage. At 45 d, arils in the varieties of the treatment with 1-MCP showed 10.8%, 30.1%, 21.1%, 13.5%, and 5.3% lower ethylene production than those of the control in sequence shown in Figure 4d. On the contrary, except in 'Mollar' and 'Tunisian soft seed', ethylene production was mostly displayed slightly higher in arils with the 1-MCP treatment than those with the control from then. At 90 d, arils of 'Malisi', 'Moyuruanzi', and 'Dongyan' with the 1-MCP treatment presented 44.5%, 11.6%, and 16.2% higher ethylene production than those of the control, respectively (Figure 4d). Overall, the treatment with 1-MCP promoted a reduction of ethylene production and, remarkably declined the peak in whole fruit of all the varieties.

### 3.4. Effects of 1-MCP Treatment on Sensory Quality

The descriptive sensory panelists evaluated overall acceptance, odor, flavor, and color of pericarp and aril at 90 d after the low-temperature storage with the 1-MCP and control treatments (Figure 5). 'Moyuruanzi' outperformed the other investigated varieties in terms of sensory properties and preferences at the end of the low-temperature storage. Regarding overall acceptance, odor, flavor, and color, 'Moyuruanzi' scored the highest, whereas 'Malisi' scored the lowest. 'Mollar' and 'Tunisian soft seed' showed clearly higher scores of overall acceptance with the 1-MCP treatment (6.10 and 5.90) than the control (4.90 and 5.10). Few changes of odor and flavor were perceived in all five varieties by 1-MCP. A better acceptance of fruit color, especially of peel color, were rather recorded in fruit with the 1-MCP treatment compared to the control. The 1-MCP treatment resulted in a pericarp color of 'Mollar', 'Tunisian soft seed', and 'Moyuruanzi' being higher by 30.6%, 23.5%, and 14.8% than those of the control, respectively. Additionally, their aril color scores differed from the control by 9.8%, 5.4%, and 12.6%, respectively. These results showed that there was no negative effect on the sensory quality of 'Malisi' and 'Dongyan', but overall acceptance and color were particularly appreciated in 'Mollar', 'Tunisian soft seed', and 'Moyuruanzi'.



**Figure 5.** 1-MCP treatment affected sensory properties of the five pomegranate varieties during low-temperature storage. Control: untreated control; 1-MCP: samples that were treated with 1-MCP stickers. All the fruit were stored at the low temperature of 4 °C with the relative humidity of 85–90%.

#### 4. Discussion

In recent years, the soft-seed pomegranate industry has developed rapidly and has become an essential pillar for poverty alleviation and rural revitalization in Yunnan, Sichuan, and Henan in China, especially with the introduction of several new varieties in recent years [14]. Nonetheless, they are all facing a limited postharvest storage life. Pomegranate fruit is prone to several physiological and chemical disorders, such as the major storage problems of water loss and browning symptoms [5–7,33]. As is common knowledge, low-temperature storage is widely used to maintain the nutritional value of fruits and vegetables. Nevertheless, studies have shown that some non-climacteric fruit can benefit somewhat from 1-MCP [23,34]. This study showed that the storage life of five pomegranate varieties treated with 1.0  $\mu\text{L L}^{-1}$  1-MCP was prolonged compared to that of the control during the low-temperature storage at 4 °C, and the fruit maintained a comparatively lower peel browning and weight loss (especially for three soft-seed varieties) as well as better fruit quality.

##### 4.1. Peel Browning

The browning of pomegranate peels represents a common problem after harvest. Although browning increases under 5 °C, storage at low temperatures is necessary [6]. Symptoms of peel browning include pitting, husk scald, some softening, a higher sensitivity to decay, internal seed discoloration, and browning of chilling injury in postharvest pomegranate fruit during low-temperature storage [29,33,35]. We observed peel browning in the three soft-seed pomegranate fruit ('Mollar', 'Malisi', and 'Tunisian soft seed') from 30–45 d and in the two semi-soft-seed fruit ('Moyuruanzi' and 'Dongyan') from 60 d after the low-temperature storage. The two types of semi-soft- and soft-seed displayed obvious differences with the storage duration (Figures 1 and 2). A similar difference among varieties was even reported on soft-seed 'Tunisia' compared with hard-seed 'Yudazi' [10], suggesting that the different responses to 1-MCP were due to different cultivar traits. Additionally, it can be connected to their various ancestries. From Tunisia and Israel, these three soft-seed types had less endurance in cold temperatures than semi-soft-seed kinds originating in China.

It is reported that 1-MCP can significantly reduce the browning of grape stalks and lychees during postharvest storage [19,21]. 'Wonderful', 'Dahongpao', and 'Tunisian' all had a lower browning index in pomegranate peels with 1-MCP treatment [5,6,27,28]. Similarly, 1-MCP did not delay the occurrence of browning, except in 'Tunisian soft seed'; however,

a significant decrease in browning index was recorded, especially in the three soft-seed varieties after the 1-MCP treatment during the low-temperature storage (Figures 1 and 2a). Malonaldehyde (MDA) levels are always elevated, and enzymatic components are always triggered when pomegranates brown [10,32,34,35]. The 1-MCP treatment had been proven to reduce the browning by inhibiting the activities of PPO (polyphenol oxidase) and POD (peroxidase) and lowering MDA levels in 'Tunisian soft seed' fruit (unpublished data). A previous report showed that decreased peel browning in 'Dahongpao' and 'Wonderful' by 1-MCP is linked to a decrease in MDA level and PPO activity and an increase in total antioxidant capacity [23,28]. Instead, higher MDA level and PPO activity but lower antioxidant capacity (ascorbate peroxidase and catalase, etc.) have been implicated in aril browning of 'Tunisia' during cold storage, peel browning of 'Baiyushizi', and superficial browning of 'Wonderful' caused by chilling injury [32,36,37].

#### 4.2. Weight Loss

Weight loss indicated by water and carbon losses and caused by transpiration, respiration processes, and a vapor pressure deficit in pomegranate is important in spite of its thick rind and tough leathery outer skin, leading to loss of ethylene gas, as well as aromatic and volatile organics [6,7,34]. The application of modified atmosphere packaging, film wrapping, and coating, such as perforated polypropylene film and acacia gum, can successfully reduce weight loss [5,34,38]. In this present study, compared to the control, retarded weight loss was recorded in pomegranate fruit of the five varieties with the 1-MCP treatment during the low-temperature storage (Figure 3f). Moreover, when weight loss is excessive, it will result in browning of the peel and arils and hardening of the rind [7,34,39].

#### 4.3. Fruit Quality

Regarding quality criteria, the color of pomegranate is an essential attribute affecting marketability, purchasability, and consumer preference [40]. Through the calculation of hue values that measure the degree of saturation of color and the evaluation by sensory panelists, a marginally improved color was shown in the studied pomegranates following 1-MCP treatment, especially in 'Moyuruanzi', in comparison to the control (Figures 2d and 5). Acidity affects the taste and color of pomegranate arils [27,41], and previous studies have reported that organic acids of fruit are substrates consumed during storage in respiratory processes [39]. Here, a prevention of the decrease in titratable acids was found in all the investigated arils with 1-MCP treatment during low-temperature storage (Figure 3b). Furthermore, 1-MCP had no effect on total soluble solids. Obviously, ratios of total soluble solid and titratable acid were undoubtedly reduced by the 1-MCP treatment (Figure 3c). Valdenegro et al. [5] indicated that no significant differences were observed in these parameters for arils of 'Wonderful' with 1-MCP or ethylene treatment during the whole period of cold storage at 2 °C, including 3 days at 20 °C. However, the application of coatings reduces the loss of titratable acids and decreases ratios of total soluble solids and titratable acids during low-temperature storage [39].

Vc is a typical non-enzymatic antioxidant substance with antioxidant and anti-aging effects [10]. As the main component of phenolics, anthocyanin is also an essential anti-aging and antioxidant substance [42]. They also affect the color of fruits [40]. An increasing trend of the anthocyanins but a decrease trend of Vc was observed in the arils of these five pomegranates during low-temperature storage. However, the 1-MCP treatment had a favorable impact on halting the decline of Vc, while it had no discernible impact on the growth of their anthocyanins (Figure 3d,e). Similar results had also been reported in 'Wonderful' treated with 1-MCP during the low-temperature storage at 2 °C and 'Rabbab-e-Neyriz' treated with coating during cold storage ( $2 \pm 0.5$  °C) for 45 d [38,39]. Furthermore, the fruit of three soft-seed varieties with the treatment of 1-MCP rather than the two semi-soft-seed varieties effectively maintained the color and total acceptance (Figure 5), further indicating that the various responses to 1-MCP were brought on by various cultivar features.

#### 4.4. Respiration and Ethylene Production

Although pomegranate is a non-climacteric fruit, it was affected by 1-MCP and analogous exogenous ethylene, indicating that ethylene might be involved in its senescence [5]. 1-MCP promotes the postharvest preservation of climacteric or non-climacteric fruits, usually by lowering or delaying the peak of respiration intensity and ethylene production [15,16]. Valdenegro et al. [5] found that exogenous ethylene treatment had only a temporary or no effect on the ethylene production of postharvest pomegranate fruits stored at 2 °C. However, Zhang et al. and Wan et al. [26,28] also demonstrated that 1-MCP may considerably reduce the rate of the pomegranate respiration or ethylene production during cold storage. Therefore, in this study, the effects of 1-MCP treatment on respiration and ethylene production of whole fruit and arils of these five pomegranates during the low-temperature storage were thoroughly examined. The results showed that 1-MCP boosted whole-fruit respiration of the soft-seed varieties at the middle storage (30–45 d), but it blocked their subsequent increase in respiration, including that of ‘Moyuruanzi’. Meanwhile, the treatment with 1-MCP triggered a reduction of ethylene production, and it remarkably declined the peak in whole fruit of all the cultivars at 30–60 d after storage, for example of ‘Malisi’, decreasing the peak of  $2.67 \mu\text{L kg}^{-1} \text{h}^{-1}$  at 60 d down to  $0.38 \mu\text{L kg}^{-1} \text{h}^{-1}$  at 90 d (Figure 4a,c).

Notably, peel browning began coinciding with increased respiration rates and reduced ethylene generation in the soft-seed whole fruit treated with the 1-MCP compared to control fruits (Figures 1, 2 and 4). According to earlier research, the enhanced respiration and increased energy charge in longan when exposed to pure oxygen are related to their reduced browning level [43]. After short-term anaerobic treatment, a sufficient energy supply reduces the peel browning of post-harvest litchi. In contrast, a shortage of energy is one of the main reasons for longan browning [44,45]. Furthermore, Valdenegro et al. [5] also observed that the pomegranate browning during cold storage is preceded by a spike in ethylene production and an increase in respiration rates and consumption of oxygen by 1-MCP. Therefore, we hypothesized that 1-MCP treatment enhanced the respiration intensity of the three soft-seed pomegranates, allowing for the redistribution of additional materials and energy charges to the peels. This could ensure requirement for resistance to halt browning processes during low-temperature storage, which might be implicated in the ethylene associated pathway induced by the 1-MCP application.

#### 5. Conclusions

Short storage life is the main problem of pomegranate fruit, indicated by peel browning, weight loss, flavor and color loss, and other symptoms. Despite its universal presence in storing and preserving fruits and vegetables, it must be enhanced when applied to various soft-seed and semi-soft-seed pomegranates. This study determined the effects of low-temperature storage and 1-MCP on peel browning and some physiological and biochemical indices of three soft-seed and two semi-soft-seed pomegranate varieties. All fruit studied with the 1-MCP treatment showed less quality degradation than those in the control, as seen by lighter peel browning, less weight loss, fewer decrease in titratable acids and Vc, and better color and acceptability. Although pomegranate is a non-climacteric fruit that always has low levels of ethylene and respiration during ripening, remarkable suppression of ethylene production peaks in all varieties and periodical increase in respiration rates were observed at 30–60 d after the 1-MCP treatment compared to the control. This was especially true in the whole fruit of three soft-seed varieties. Noticeably, this behavior began when 1-MCP started to reduce peel browning, if not earlier. Collectively, our research showed that 1-MCP positively impacted pomegranate fruit quality during the low-temperature storage, which would serve as an important theoretical foundation for postharvest practicality on pomegranate fruit in China.

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