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Effects of Different Treatments on Physicochemical Characteristics of 'Kyoho' Grapes during Storage at Low Temperature

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Abstract: Low temperature storage is widely used to maintain the postharvest quality of table grape. However, grape clusters easily undergo deterioration without treatment during the storage time. The main goal of this study was to evaluate the effect of postharvest 1-methylcyclopropene (1-MCP), calcium chloride (1%) and ethanol (16%), and the combination of 1-MCP with calcium chloride and ethanol treatments on maintenance of quality of table grapes 'Kyoho' (*Vitis vinifera* × *Vitis labrusca*) under 5 °C and 0 °C storage. Changes in decay incidence, weight loss, rachis browning and quality indexes of grape clusters were investigated. The results were as follows: all treatments significantly reduced the decay incidence, weight loss, rachis browning at both low temperatures storage; 1-MCP had positive effect for reducing the decay incidence in early stage, but no effect in late stage; there are no significant variations of taste and color quality indexes under two low temperatures storage, regardless of the treatments. Overall findings suggested that the combination of 1-MCP with calcium chloride and ethanol treatment is suitable for short-term 0 °C storage, while for long-term 0 °C storage, calcium chloride (1%) and ethanol (16%) treatment should be selected.

Keywords: *Vitis vinifera* L.; storage; 1-MCP; calcium chloride; ethanol

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1. Introduction

Grapevine (*Vitis vinifera* L.) is one of the most important economically fruit crops cultivated worldwide. Approximately 90% of production is used for fresh fruit in China [1]. Traditionally, table grapes have been regarded as a highly perishable and non-climacteric fruit with low physiological activity, but they are subject to postharvest losses such as decay, water loss and rachis browning after harvest and during long-term storage [2–4]. As the fruit market becomes more competitive, consumers demand high quality. With regard to table grapes, due to the impact of consumer perceptions and the fruit market prices, grape quality has attracted more and more attention from producers and exporters. Thus, table grape producers are constantly searching for new technologies to maintain the fresh appearance of grape clusters once harvested [5]. In some cases, vineyards are located far away from the market. Therefore, innovative technologies are needed to focus on retarding or inhibition of the physicochemical changes occurring during long-term storage of berries picked with optimal quality parameters.

So far, low temperature storage is the most widely used postharvest technology to maintain the fruit quality and other horticultural products, and prolong their shelf-life [6,7].

However, the length of storage of table grape is reported to be limited under low temperature, and the commercial value decreases without appropriate treatments [8,9]. Thus, different postharvest treatments combined with low temperature storage have been used to maintain table grape quality. The use of sulfur dioxide (SO_2) is the most common commercial method for maintaining quality and prolong postharvest life in grape clusters. Moreover, the application of controlled atmospheres with a high CO_2 content under a continuous flow has been proven to be an effective tool for controlling postharvest diseases and prolonging low storage time in table grapes [5,9]. However, the application of atmospheres hard to control, and SO_2 is strictly limited in many countries because its residues are harmful to human health and might cause phytotoxicity symptoms on fruit [3,8,10]. Growing producer and consumer awareness of the potential hazard of chemical treatments during postharvest storage has led to the development of safe treatments for this purpose in horticultural produce. Therefore, more environmentally friendly and harmless treatments should be developed as substitutable technologies for storage of table grapes [10,11].

As a substitutable technology, 1-MCP is being considered as an ethylene inhibitor; it has been used to block the ethylene binding to the receptor, preventing down-stream ethylene signal transduction at a very low concentration for delaying fruit ripening and improving storage quality of many horticultural products [12–15]. It has been found that 1-MCP alone or combined with other preservation methods could extend the storage period of fruits, such as grape [12], banana [13], blueberry [16], fig [17], apple [18], persimmon [19], and so on. Ethanol is a common and cheap food additive with antimicrobial activity. Ethanol vapor or dip treatment has been proved to be effective in inhibiting postharvest fungal infections in many fruits [20,21]. However, it is difficult to contain an appropriate concentration of ethanol vapor in the manned workplaces, which reduces its feasibility and limits its practical use. Moreover, high ethanol concentration may lead to increased treatment costs and cause environmental and safety problems, as well as osmotic damages in fruit tissue [20]. Application of ethanol by dipping is an economic and safe postharvest treatment technology. It has been shown to effectively improve storage life of table grapes, mainly by limiting postharvest rot development [22,23]. In addition, calcium is a secondary messenger that plays a positive role in regulating physiological functions and maintaining fruit storage quality [24,25]. Previous studies have found that spraying calcium can improve the resistance of grape berries to abiotic/biotic stress and prolong their shelf life [24]. Nigro et al. [26] found that applications of a 16% ethanol solution, containing 1% $CaCl_2$, reduced rotten clusters. Moreover, calcium chloride postharvest treatment may delay apples softening and reduce physiological disorders incidence [25].

'Kyoho' grapes (*Vitis vinifera* × *Vitis labrusca*) are famous for their crisp texture, large sized berries, and a good ratio of sugar to acid, and are one of the most important commercial grape varieties in China. However, grape clusters can deteriorate rapidly without appropriate treatments after harvested [27]. Therefore, the aim of our present work was to evaluate the effect of 1-MCP, calcium chloride and ethanol, and the effect of combined 1-MCP with calcium chloride and ethanol treatments for maintaining postharvest quality and prolonging the storage time of grape berries. Our study aimed to develop an economic and safe postharvest technology for quality maintenance of table grapes.

2. Materials and Methods

2.1. Materials and Treatments

Table grapes 'Kyoho' (*Vitis vinifera* × *Vitis labrusca*) were obtained in 16 August 2013 from a vineyard located in Ningbo, Zhejiang Province, China. Grape clusters were harvested manually at commercial maturity, and then were transported to the laboratory immediately. The grape clusters were selected on the basis of uniform size, color, firmness, and the absence of wounded and moldy were randomly distributed into batches before the postharvest experiment. Approximately 5 kg berries were randomly selected as the initial sampling point for storage; the other berries were pre-cooled in a 5 °C cold storage for 12 h. On the second day, the pre-cooled berries were randomly divided into 4 group experiments

(each experiment was repeated three times) for the following treatments: (1) 1-MCP: grape clusters were completely immersed in 1 µg/kg of 1-MCP aqueous solution for 5 min and then taken out for air drying; (2) Calcium chloride and ethanol: grape clusters were uniformly sprayed with 16% aqueous ethanol containing 1% calcium chloride; (3) Combined 1-MCP with calcium chloride and ethanol: grape clusters were completely immersed in 1 µg/kg of 1-MCP aqueous solution for 5 min and taken out for air drying, then uniformly sprayed with 16% aqueous ethanol containing 1% calcium chloride; (4) Control: without any treatment. According to the above treatment, about 3 kg of each sampling point for each treatment were weighed, then carefully and neatly put into open packing box in one layer. Grape berries in each treatment were further divided into two groups, stored in cold storage at 0 °C and 5 °C in humidity of approximately 85–95%.

2.2. Measurement of Decay Incidence, Weight Loss, Rachis Browning and Firmness

Grape berries were withdrawn randomly at 15, 30, 45, 60, and 75 days in two temperature storage in order to determine the biochemical changes that had occurred. Five grape clusters in each group were used for the statistics of decay incidence, weight loss, and rachis browning. Decay incidence: Decay incidence was quantified in each sampling point by calculating the percentage of rotten grape berries independently in each treatment. Weight loss: Grape berries were weighed at the beginning of the experiment, and thereafter every half a month during the storage time. Weight loss was expressed as the percentage loss of the initial total weight. Rachis browning: Rachis browning was assessed using the following subjective scale based on previous studies [9,28]: (0) none, entire rachis including the cap stems, green; (1) slight, cap stems showing browning, but the browning area is less than a quarter; (2) moderate, cap stems and rachis showing browning, and the browning area accounts for a quarter to a half; (3) severe, the browning area of cap stems and rachis is more than a half but less than three quarters; (4) extreme, the browning area of cap stems and rachis is more than three quarters. The rachis browning was expressed as a percentage of rachis area. The firmness was measured using Ta-xt2i Plus texture analyzer (Stable Micro System, UK) with a flat probe 2 mm in diameter. A total of 30 berries were selected randomly in each treatment at every sampled point.

2.3. Color and Total Soluble Solids (TSS) Analysis

Color and TSS were measured and sampled at each sampling point. All samples were separated by skins and pulp, frozen in liquid nitrogen immediately, and stored at −80 °C for future use. All experiments were analyzed with three biological replicates. Color measurement was carried out at two distributed opposite sites of each fruit by a Hunter Lab Mini Scan XE Plus colorimeter (Hunter Associates Laboratory, Reston, VA, USA) following our lab methods [29]. A total of 30 berry repetitions were done for each treatment. TSS contents were measured with a refractometer PR101-a (Atago, Tokyo, Japan) following the manufacturers' protocols with 30 berries for each treatment, and each berry had two measurements.

2.4. Total Phenolics, Total Anthocyanins and Total Procyanidins Analysis

The grounded powder of 1 g was extracted with 4 mL of 70% aqueous ethanol (containing 1% formic acid) for three times. The supernatants of three extractions were combined for the determination of total phenolics, total anthocyanins, and total procyanidins. Three biological repetitions were done for each treatment. Total phenolics were determined according to our previous method [29] with slight modification. Approximately 0.5 mL of extracts with 4 mL of ddH_2O was placed in a test tube, then 0.5 mL of 0.5 N Folin–Ciocalteu reagents were added to react for 3 min, then neutralized with 1 mL of saturated Na_2CO_3, followed by 2 h incubation at 30 °C. Absorbance at 760 nm was read using a spectrophotometer (DU-8000 Beckman Coultor, USA). The content of total phenolics was calculated as gallic acid equivalent. Total anthocyanins were measured according to our lab method [30]. The aqueous ethanol extract was diluted with 0.2 mol/L KCl buffer (pH 1)

or 0.2 mol/L $C_2H_3NaO_2$ buffer (pH 4.5) at a ratio of 1:4. After 20 min under darkness, absorbances at 510 nm and 700 nm were measured at both pH. Results were expressed ascyaniding-3-glucoside equivalent using a molar extinction coefficient of 29,600. Total procyanidins were determined according to our previous method [29]. Approximately 50 µL of extracts with 250 µL of 4-dimethylaminocinnamaldehyde solution (hydrochloric acid and ethanol; 1:9 v/v) to react for 15 min under darkness, absorbance at 640 nm was measured using a microplate reader (Thermo, Waltham, MA, USA). The results were expressed as mg procyanidin B2 equivalent/g of fresh sample.

2.5. Quantification of Individual Sugars

Sugar samples were extracted and derivatized according to [31] with slight modification. Briefly, 0.1 g of frozen pulp powder and mixed with 1.4 mL of HPLC-grade methanol, followed by being vortexed and incubated at 70 °C. After 15 min, the mixture was centrifuged at 11,000× g for 10 min. The supernatant was collected and added 1.5 mL of Millipore water containing 750 µL of HPLC grade chloroform, then spun down at 2200× g for 10 min. The upper phase of 100 µL was separated and 0.2 mg/mL of ribitol was added as an internal standard. After being fully dried, all samples were derivatized by adding 60 µL of methoxyamine HCl in pyridine (20 mg/mL), then vortexed and incubated at 37 °C for 90 min. After that, 40 µL of MSTFA + 1% TMCS were added, vortexed, and incubated at 37 °C for 30 min. The derivatized samples were analyzed using Agilent 7890 Gas Chromatography (GC) coupled to 5975 MSD scanning. Approximately 1 µL of sample was injected at a split ratio of 10:1, and separated by fused-silica capillary column (60 m × 0.25 mm i.d., 0.25 µm HB-5MS stationary phase). The inlet and transfer line were held at 250 °C. The flow rate was set as 1.0 mL /min. Temperature program: 100 °C for 1 min, ramped at 3 °C/min to 184 °C, increased to 190 °C at 0.5 °C/min, increased to 250 °C at 10 °C/min, held for 1 min, increased to 280 °C at 5 °C/min, and then held for 3 min. Absolute quantification of the individual sugars was analyzed as standards with three biological repetitions.

2.6. Statistical Analysis

The statistical analyses were analyzed using SPSS version 16.0 statistical software package (IBM, Armonk, NY, USA), and presented as mean ± standard error (SE). The statistical significance of differences ($p < 0.05$) was determined by Student t-test. Origin 8.0 (Microcal Software Inc., Los Angeles, CA, USA) was applied for figures construction.

3. Results

Decay is one of the most important reasons for the postharvest loss of grape berry. In present study, the decay incidence was calculated during the period of storage until it was more than 50%. As shown in Figure 1a, the decay incidence during the storage increased with the elongation of storage time. It is obvious that the decay incidence of grape berry at 0 °C was significantly lower than 5 °C throughout the whole storage period, which indicates that low temperature has a good effect on reducing the decay of the grape berry. During the first month of storage, the decay incidence remained low, and the decay incidence of all treatments was significantly lower than that of the control group at the two storage temperatures. After a month, the decay incidence increased significantly, especially for those treated with 1-MCP. Within two months, the decay incidence at 5 °C under each treatment was more than 50%, leading to the end of the experiment at this storage temperature. At the end of the 5 °C storage, with significant differences among the treatment, the lowest and highest decay incidence were obtained from combined 1-MCP with calcium chloride and ethanol (10.95%) and control (25.32%) treatments, respectively. At storage temperature of 0 °C, the decay incidence increased dramatically in every treatment after more than two months, especially for those in the control group. Interestingly, in the second month of storage, the decay incidence varied significantly between treatments, from lowest to highest is calcium chloride and ethanol (12.31%), combined 1-MCP with calcium

chloride and ethanol (24.57%), 1-MCP (33.5%), and control (40.01%). On the 75th day, at the end of storage at 0 °C, the decay incidence of calcium chloride and ethanol (17.36%) treatment was significantly lower than that of the 1-MCP (50.76%) and control (51.72%) groups, indicating that 1-MCP had a positive effect in the early stage, but no effect in the late stage on the postharvest grape berries.

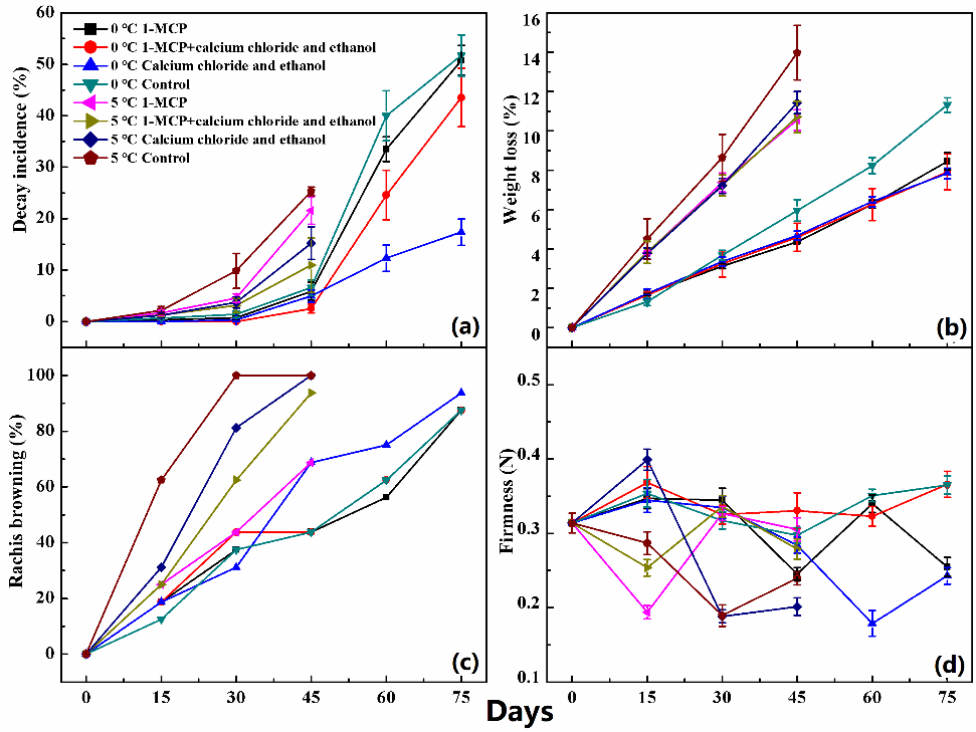

Figure 1. Dynamic changes of decay incidence (**a**), weight loss (**b**), rachis browning (**c**), and hardness (**d**) of grape berry.

In the current study, storage duration and treatment significantly influenced weight loss of grape berries at both 0 °C and 5 °C (Figure 1b). The weight loss rate of grapes increased significantly ($p < 0.05$) with the extension of storage time. Among the treatments, there was no significant difference between 1-MCP, calcium chloride, and ethanol, and the combination of 1-MCP with calcium chloride and ethanol treatments ($p > 0.05$), but they had significantly ($p < 0.05$) better effects on reducing the weight loss rate of grape berry than the control. At the end of storage, the highest weight loss rate was found in control group (13.97%), while the lowest weight loss rate (10.55%) occurred in 1-MCP application at 45th day of 5 °C storage; and the highest weight loss rate was found in control group (11.32%), while the lowest weight loss rate (7.83%) occurred in calcium chloride and ethanol treatment group at 75th day of 0 °C storage. Additionally, the weight loss rate of the grape berry stored at 0 °C was much lower than that at 5 °C.

As shown in Figure 1c, the rachis browning rate increased markedly in the control group from 62.5% browning after 15 days to totally browning at 30 days of 5 °C storage. In contrast, rachis browning rate in calcium chloride and ethanol treatment group reached 100% at 45 days of 5 °C storage. Furthermore, grape berries treated with 1-MCP showed significant lower values for rachis browning rate compared with other groups at the end of 5 °C storage. However, the rate of rachis browning rate increased with the storage time at 0 °C, and there was no effect under these treatments. As for the firmness of the berry, this fluctuated irregularly during the storage, in a range of 0.18 N–0.37 N. Significant differences were seen in some time points. However, we are inclined to believe it is due to the individual difference, considering that no significant regularity was found in the whole storage period (Figure 1d).

TSS is an important index for the evaluation of taste quality of grape berries. In the current study, the TSS was measured during the whole grape berry storage period. The results showed that newly harvested grape berry had a TSS value of 18.6%, and tended to increase slightly during the storage time at both temperatures. At the end of 5 °C storage, the TSS values in every group were more than 20%, and there was significant difference ($p < 0.05$) compared with newly harvested grape berry but no significant difference between treatments ($p > 0.05$). At the end of 0 °C storage, the TSS value of grape berries in the control group was lower than that of the other three treatment groups (Figure 2a).

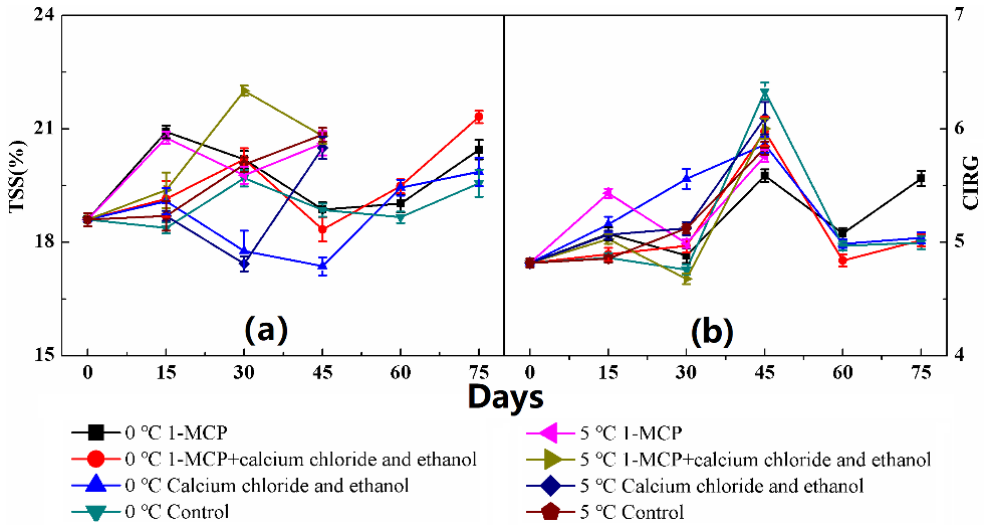

Figure 2. Dynamic changes of TSS (**a**) and CIRG (**b**) value of grape berry. The data is presented as mean ± standard error (SE) of at least three repetitions.

In the present study, chromatic aberrations were measured according to the CIE 1976 L * a * b * color scale throughout the storage period (Figure 2b). Our results showed that the color index of red grapes (CIRG) value increased slightly with the storage time, and there was no significant difference between treatments ($p > 0.05$) at the end of 5 °C storage, while the CIRG value in 1-MCP treatment group was significantly higher than that of other groups.

The effect of 1-MCP, calcium chloride, and ethanol, and the combination of 1-MCP with calcium chloride and ethanol treatments on the total anthocyanins, total phenolics, and total procyanidins contents in grape skins during the storage period at two temperatures are shown in Figure 3a–c. Results have shown that all the total anthocyanins, total phenolics, and total procyanidins contents of grape skins were insignificant, and there was no obvious pattern to follow for the increase and decrease over the entire storage period, regardless of the treatments and temperatures.

Soluble sugars were determined in grape pulp (Figure 4a,b), from which it was found that the glucose and fructose were the prominent sugars in grape. The contents of glucose maintained stable, and the contents of fructose increased slightly throughout the storage process in all treatments in grape pulp, but no significant difference was observed among different treatments, regardless of the temperatures.

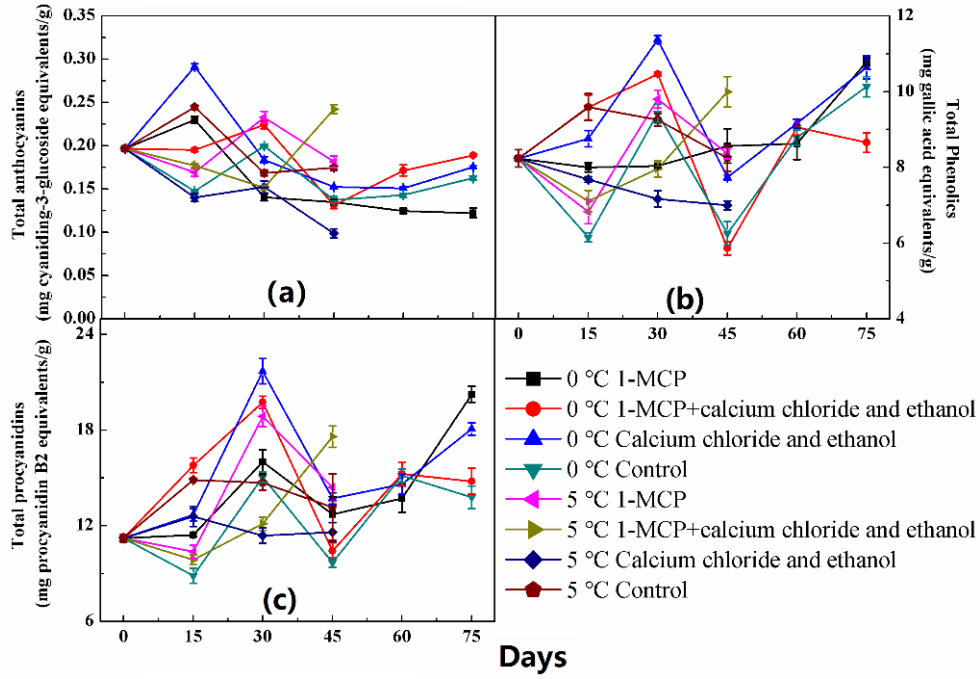

Figure 3. Dynamic changes of total anthocyanins (**a**), total phenolics (**b**), and total procyanidins (**c**) value of grape skin. The data is presented as mean ± standard error (SE) of at least three repetitions.

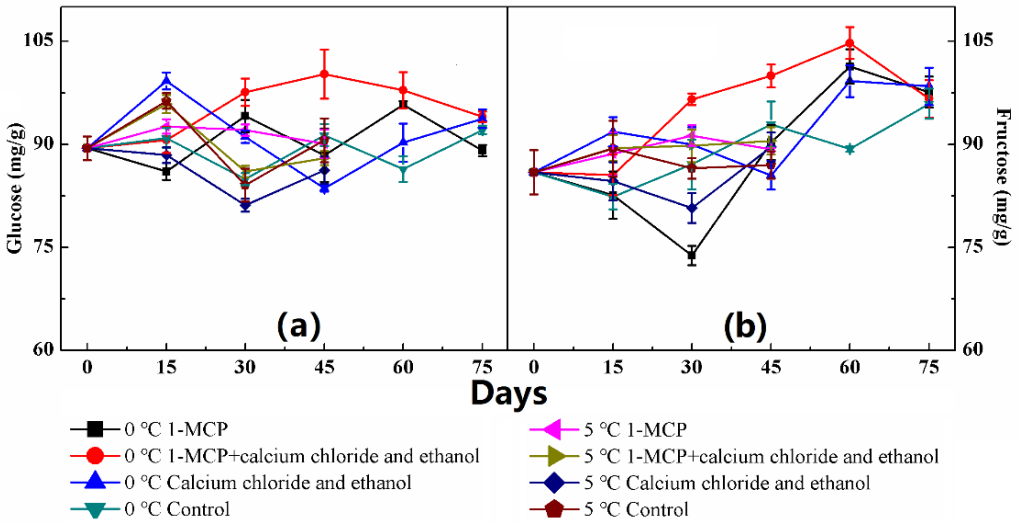

Figure 4. Dynamic changes of glucose (**a**) and fructose (**b**) value of grape pulp. The data is presented as mean ± standard error (SE) of at least three repetitions.

4. Discussion

The postharvest table grape berries undergo a process of quality deterioration, which experience decay, weight loss, rachis browning, and so on. With the elongation of storage time, the quality deteriorates more. Moreover, inappropriate postharvest treatments accelerate the quality deterioration process [9,32]. To compare the effects of several harmless treatments on table grape storage, we performed decay and fruit quality assays of grape under different treatments at both low temperatures.

It is well known that gray mold infection (*Botrytis cinerea*) leading to berry decay is the most critical problem for the postharvest loss of grape [3,8,33]. 1-MCP has successfully been identified in various climacteric and non-climacteric fruits to extend the storage time and maintain the quality of products [13,14]. It is an effective and inexpensive substance, which acts at very low concentration (2.5 nL/L–1 µL/L) to inhibit ethylene

signal transduction and slow postharvest vitality [18,34]. Furthermore, 1-MCP inhibits activities of polygalacturonase, pectinesterase, and cellulose, decreases disassembly of cell wall polysaccharides, and protects the berries from pathogen infection, contributing to decrease decay during storage periods [12]. In the present study, our results have shown that the application of 1-MCP, calcium chloride, and ethanol contributed to the quality maintenance of 'Kyoho' berry during storage at both low temperatures. It is worthwhile to note that 1-MCP significantly inhibited grape berry decay incidence during the early stage of the postharvest storage, which was similar to previous studies [12,35]. Ethanol dips and vapors have been identified to inhibit postharvest fungal infections [21]. Moreover, calcium can inhibit pathogen pectolytic enzyme activities for defending against plant pathogens [36]. In the previous studies, Lurie et al. [37] found ethanol (10–20%) effects in reducing decays in table grape. Additionally, Nigro et al. [26] showed preharvest sprays 1% $CaCl_2$ was efficient to control postharvest decay in grapes. Treatments with a low concentration of ethanol or $CaCl_2$ maintained the quality of the grape, indicating the effectiveness of these types of ethanol or $CaCl_2$ treatment. It was determined by a series of experiments testing aqueous sprays that a 16% ethanol combination with 1% $CaCl_2$ was the best dose for limiting fungal development in table grapes [26,38]. Similar results were also observed in our study. Our results demonstrated that a spray of 16% aqueous ethanol containing 1% calcium chloride uniformly sprayed on grape clusters has the best effect to decrease decay during long-term grape storage periods. This work is also in agreement with the result that the physiological process of fruit is slower and the pathogenicity of pathogens is weaker at low temperature [3], resulting in a relatively lower decay incidence at 0 °C as compared to 5 °C.

Weight loss is a simple and objective measure to evaluate the response of horticultural products to treatments [10]. Weight loss is mainly due to water evaporation in fruit caused by transpiration and respiration processes during storage periods [39]. 1-MCP has been demonstrated significantly reduced respiration, which leads to reduced water loss [15]. Published information show postharvest calcium treatment leads to changed gas diffusion rates, resulting in the inhibition of respiratory metabolism [36]. Additionally, calcium, a constituent of the cell wall, plays an important role in influencing cell wall strength for retarding the weight loss of grape [10]. In the present study, it has shown that 1-MCP, calcium chloride (1%), and ethanol (16%), and combined 1-MCP with calcium chloride and ethanol treatments significantly retarded weight loss. Furthermore, compared with 5 °C, weight loss of grape was less in all treatments at 0 °C. Rachis browning is one of the main factors that reduces the quality and affects the marketing of table grape clusters during storage at low temperature [9]. In general, rachis browning has been associated with water loss and senescence [5]. Our finding agreed with the report that postharvest exposure to 1-MCP had significant effects on rachis respiration and ethylene production rates for delaying rachis browning [15]. In the present study, we also found low concentrations of calcium chloride and ethanol spray can significantly delay rachis browning, most probably owing to calcium protective effects on the cell wall, as well as delay senescence, thus delaying water loss.

Pectin and cellulose are considered as large proportion of the polysaccharide components, that affects the hardness of grape berries [40]. In our study, the firmness remained stable with slight fluctuation during the storage. This result indicated that the treatments in present study had little effect on the pectin and cellulose changes of 'Kyoho' table grapes.

In general, consumers judge the grape quality based on taste, color, and firmness. The main challenge facing producers of grape is the maintenance of postharvest quality. TSS is an important index for the evaluation of taste quality of grape. The sugars (mainly glucose and fructose in grape berry) are essential for maintaining the basic function of the fruit, and contributing to its taste [41,42]. However, phenolic compounds play important roles in the appearance of fruits [1]. Among them, anthocyanin is responsible for the color of grape berries, and procyanidin plays critical roles in the bitterness, aroma, and astringent properties of grape [43]. In the present work, we have found that no significant variations in

such attributes (TSS, CIRG, total anthocyanins, total phenolics, total procyanidins, glucose and fructose, and firmness) during both low temperature storage periods, regardless of the treatments. Low temperature storage is used to maintain the grape postharvest quality because the physiological process is slower and the pathogenicity of pathogens is weaker [3]. Ethanol and $CaCl_2$ can control or reduce certain physiological disorders, and reduce the incidence of fungal pathogens in many fruits [26]. 1-MCP can be used to delay fruit ripening and improve storage quality by inhibiting ethylene production [12]. We infer that these treatments in our experiment could be used alone or in synergy with others to maintain grape postharvest quality.

5. Conclusions

In conclusion, our results provide evidence that 1-MCP, calcium chloride, and ethanol, as well as the combination of 1-MCP with calcium chloride and ethanol treatments, could significantly reduce the decay incidence, weight loss, and rachis browning, and maintain grape quality during low temperature storage. Generally speaking, low temperature storage, especially 0 °C, is the most optimal treatment to maintain the postharvest quality of grapes. Our findings suggested that the combination of 1-MCP with calcium chloride and ethanol treatment should be better used for short-term table grape storage at 0 °C, but for long-term storage, a calcium chloride and ethanol treatment should be chosen, and still be stored at 0 °C.

Author Contributions: Experiment design, F.L. and C.S.; experiment process, F.L., C.W., L.S. and P.L.; data analysis, F.L., J.C., C.Z. and Y.W.; reagents, materials and analytical tools, F.L. and C.S.; writing—review and editing, F.L. and C.S. All authors have read and agreed to the published version of the manuscript.

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