Effects of inclusion compounds of 1-methylcyclopropene/α-cyclodextrin or 1-methyl-3-(2-methylcyclopropyl)-1-cyclopropene/Cu-β-cyclodextrin on the preservation of sweet cherry (*Prunus avium* L.)

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Sweet cherry (*Prunus avium* L.) was used to study the effects of 500, 1000 and 2000 nl/l 1-methylcyclopropene (1-MCP; released from the inclusion compound of 1-methylcyclopropene/α-cyclodextrin; 1-MCP/α-CD) and 800, 1600 and 3200 nl/l 1-methyl-3-(2-methylcyclopropyl)-1-cyclopropene (1-MMCPCP; released from the inclusion compound of 1-methyl-3-(2-methylcyclopropyl)-1-cyclopropene/Cu-β-CD; 1-MMCPCP/Cu-β-CD) on storage quality at ambient temperature after 8 h of treatment. UV spectra indicated both 1-MCP and 1-MMCPCP distinctly slowed down the increasing rate of browning level and the decreasing rate of titratable acidity content, soluble protein content, superoxide dismutase activity and ascorbate peroxidase activity of sweet cherry. However, they had little control on the decrease in soluble solids content. As a whole, the quality of treated sweet cherry was preserved much better than that of the controls. Moreover, a comparison between 1-MMCPCP/Cu-β-cyclodextrin and 1-MCP/α-cyclodextrin showed that the preservation effects of these two inclusion compounds were similar.

Keywords: Cyclodexdrin, inclusion compounds, preservation, sweet cherry.

THE ethylene receptor in plant tissue is cannot expressed by 1-methylcyclopropene (1-MCP), which competes with ethylene for binding sites[1]. Owing to the established role of ethylene in the treatment and storage of the vast majority of harvested fruits and plants, whether beneficial or harmful, the reactions between 1-MCP and post-harvest biology are understandable processes[2]. 1-MCP has been used for post-harvest experiments on fruits and vegetables, including broccoli[3,4], parsley leaf[5], green asparagus[6], lettuce[7], cucumber[8], persimmon[9,10], apple[11,12], tomato[13], avocado[14,15], pear[16], plum[17,18] and guava[19,20]. The scientific results indicate that 1-MCP can control ripening and senescence in the harvested fruits and vegetables, thus highlighting its potential commercial applications. Climacteric fruits have become the main target for the study of 1-MCP, and the response of these fruits has proved that 1-MCP is the opposite to ethylene. Studies on non-climacteric fruits showed that the maturation processes are ethylene-dependent and ethylene-independent, and presented interesting problems on the typical differences between climacteric and non-climacteric fruits[2]. Besides 1-MCP, more effective ethylene antagonists like 1-MCP-related compounds have also been studied. Cyclopropene and its derivatives are also effective antagonists which inactivate the receptors[21]. In the earlier studies, cyclopropene, 1-MCP and 3,3-dimethylcyclopropene were used[21], which had vastly different properties[13,22]. 1-Methyl-3-(2-methylcyclopropyl)-1-cyclopropene (1-MMCPCP), which is a cyclopropene derivative, also has impressive effects of delaying the ripeness on the preservation of sweet cherry.

Sweet cherry has high market demand. Unfortunately, fresh cherries have a very short consumption period compared to other early seasonal fruits, and their taste and life is limited by this factor. Ethylene does not obviously affect post-harvest shelf life of some non-climacteric fruits such as sweet cherry and grapes[23]. Although it is non-climacteric, sweet cherry fruit has undergone similar biochemical changes. Therefore, it is possible that 1-MCP or 1-MMCPCP may have beneficial effects on the shelf life and quality of sweet cherry.

Here, sweet cherry was selected to study the effects of gaseous 1-MMCPCP or 1-MCP released from the new inclusion compounds of 1-MMCPCP/Cu-β-cyclodextrin or the inclusion compounds of 1-MCP/α-cyclodextrin on its shelf life and quality, as well as the physiological and biochemical characteristics. The aim of this study is to compare the preservation performance of sweet cherry using 1-MMCPCP/Cu-β-cyclodextrin and 1-MCP/α-cyclodextrin.

Sweet cherry (at commercial maturity) was harvested in the third week of May 2016 from a local orchard in Xi'an, Shaanxi Province of China and transferred to the laboratory in 1 h. Then the fruits were sorted to uniform size and the decayed or cracked ones were removed. After sorting, the fruits were randomly seperated into seven groups, each of 1.5 kg, and each group was then separated into three replicates and treated for 8 h at ambient temperature in an airtight container (Table 1). Required concentrations of 1-MCP (1-MCP/α-CD inclusion compound powder, 3.5 wt% active ingredient; 1-MMCPCP/Cu-β-CD inclusion compound powder, 2.36 wt% active ingredient) were prepared in the laboratory[24–26]. After 8 h, all the fruits were transferred into seven boxes at ambient temperature.

The cherry juice was extracted using a common juicer. The absorbance of the cherry juice was measured at 420 nm using a UV–Visible spectrophotometer (Agilent 8453) to determine the browning index (BI)[27]. Higher

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Table 1. Treatment of concentrations of each compound for sweet cherry

Compound	Concentration (nl/l)	1-MCP or 1-MMCPCP/Cu-β-CD released from two different kinds of inclusion compounds
CK	0	Without 1-MCP treatment
1-MCP500	500	1-MCP/α-CD inclusion compounds
1-MCP1000	1000	1-MCP/α-CD inclusion compounds
1-MCP2000	2000	1-MCP/α-CD inclusion compounds
Cu800	800	1-MMCPCP/Cu-β-CD inclusion compounds
Cu1600	1600	1-MMCPCP/Cu-β-CD inclusion compounds
Cu3200	3200	1-MMCPCP/Cu-β-CD inclusion compounds

values in absorbance correspond to higher browning of the tissue.

Sweet cherry (approximately 10 g) was juiced for determining soluble solids content (SSC). The SSC of juiced flesh was determined by Abberefractometer (WZS1). Titratable acidity (TA) was determined by titration to pH 8.1 with 0.1 M NaOH, using 25 ml sweet cherry juice in 25 ml distilled H_2O. The results are expressed as gram-malic acid per litre.

Determination for soluble protein content was used by the method of Bradford (coomassie blue staining)[28]. The protein concentration was determined using colorimetric determination by Bradford. The values were calculated by graphic interpolation on a calibration standard curve with serum albumin (BSA) at 595 nm with a UV–Visible spectrophotometer (Agilent 8453).

All enzyme tests were done at the indicated wavelengths using a UV–Visible spectrophotometer (Agilent 8453). The total activity and specificity of superoxide dismutase (SOD) and ascorbate peroxidase (APX) were expressed as nmol/min/g fresh weight and nmol/min/μg protein respectively.

SOD activity was determined according to Madamanchi *et al.*[29]. The mixture contained 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 0.1 mm EDTA, 75 μm nitroblue tetrazolium (NBT) and 1 μg protein. The reaction volume with riboflavin (2 μM) was 1 ml, and the reaction was started by illuminating the pipe under fluorescent lamp. By turning off the fluorescent lamp, the reaction stopped after 15 min. Samples covered with aluminum foil were used as non-illumination blanks. The ability of SOD to inhibit the photochemical reduction of NBT (molar extinction coefficient, 15 mM^{-1} cm^{-1}) was determined using a UV–visible spectrophotometer (Agilent 8453). The NBT reduction was calculated using its molar extinction coefficient.

APX activity was determined by the correlation decomposition of H_2O_2 ascorbic acid (molar extinction coefficient, 2.8 mM^{-1} cm^{-1}) as described by Rao *et al.*[30]. The decomposition of H_2O_2 was determined using a UV–Visible spectrophotometer (Agilent 8453). The 1 ml solution contained 100 mM potassium phosphate buffer (pH 7.5), 0.5 mm ascorbic acid and 1 mM H_2O_2. The reaction was started by adding 10 μg protein.

Each experiment from the same harvest was replicated three times to reduce experimental errors. Data were analysed statistically and the error bars depicted 95% confidence interval. (Each data point is the average of three independent samples. Vertical bars represent standard deviation of the mean.)

Sweet cherry deteriorates quickly after harvest and cannot reach the consumers with optimum after transport and marketing. The main causes of sweet cherry spoilage are weight loss, colour change, softening, surface pitting, stem browning and acidity decline, while the change in total soluble solids (TSS) is less[31]. Special attention is needed on decay, mainly as a result of species of the genera *Penicillium*, *Botrytis* and *Monilia*[32]. This fungal spoilage can cause huge economic loss, although it is reported that during harvesting decay and its effect on the quality of sweet cherry depend on the variety and ripening stage[33,34]. The change of colour is an important index of maturity and quality of fresh cherries. The development of red colour is a mature indicator, and the transition from crimson to purple occurs during ripening and can be used to predict the grade of sweet cherry.

As shown in Figure 1, the browning level of control increases sharply during postharvest ripening at day 8. The control fruits showed 50% increase at day 12 compared to that at day 0. However, increase of the brown stain was significantly inhibited during the storage period after treatment with 1-MCP/α-CD or 1-MMCPCP/Cu-β-CD of inclusion compounds. Cherries treated with the inclusion compounds of 1-MCP/α-CD showed similar trend to those treated with the inclusion compound of 1-MMCPCP/Cu-β-CD, indicating slower senescence. The brown stain of fruits from all treatments increased slowly during storage from day 6 to day 12. The brown stain from fruits treated with inclusion compounds of 1-MMCPCP/Cu-β-CD at the concentration of 800 nl/l was maintained at the lowest level at day 12. Postharvest treatments with the inclusion compounds of 1-MCP/α-CD and 1-MMCPCP/Cu-β-CD, did not cause any obvious changes in the brown parameters during storage on comparison with the control fruits. An important finding of this study was the consistently low brown staining observed in fruits treated with the inclusion compounds of 1-MCP/α-CD and 1-MMCPCP/Cu-β-CD, and the treating

effects had nothing to do with the treating concentration of the two compounds.

There were no distinct differences in the SSC between control cherries and those treated with the inclusion compounds of 1-MCP/α-CD or 1-MMCPCP/Cu-β-CD after postharvest treatments during extended storage (Figure 2). In general, SSC changed between 9% and 10.5%. As mentioned earlier, postharvest treatment with 1-MCP did not result in any significant change in the level of soluble solids in cherries[35]. Ethylene had no effect on the content of soluble solids in non-climacteric fruits[36].

As shown in Figure 3, TA of the control fruits showed a reduction of about 60% within 12 days after harvest. In contrast on treatment with inclusion compounds of 1-MCP/α-CD or 1-MMCPCP/Cu-β-CD in the first six days, the decrease in TA was significantly inhibited. Thereafter, TA began to decrease and reached different levels, being higher than those of the control fruits. The best concentration was 2000 nl/l in the inclusion compounds of 1-MCP/α-CD and 1600 nl/l in 1-MMCPCP/Cu-β-CD, whose values at day 12 were higher than that of the control fruits by 73% and 82% respectively. These results demonstrate that the inclusion compounds of 1-MMCPCP/Cu-β-CD have similar effects as those 1-MCP/α-CD on delaying the decline in TA in sweet cherry during postharvest ripening. The effects of 1-MCP or 1-MMCPCP on TA are complicated, some crops are affected badly whether others not. 1-MCP delayed TA loss in plums[37], inhibited ethylene-induced acidity loss in carrots[3], totally prevented TA loss in tomatoes[38], and maintained TA in 'Red Delicious', 'Granny Smith', 'Fuji', 'Jonagold', 'Ginger Gold' and 'Gala' apples[11,39,40]. Watkins *et al.*[41] observed that in the 1-MCP-treated fruits during air storage, like 'Law Rome', 'Delicious', 'Empire' and 'McIntosh', TA was always higher. In contrast, 1-MCP had no effect on TA of apricot[37] or 'Red Chief' apples[42] during storage at several temperatures. 1-MCP did not affect TA content of 'Shamouti' oranges[43].

Sweet cherry has a very low protein content compared to other fruits. Typically, protein content reduces from the initial levels during storage (Figure 4). The protein level in the control sweet cherry decreased significantly from 40 mg/g on day 0 to 18 mg/g on day 12. Overall, a 55% decrease in protein levels was found in control sweet cherry. Fruits treated with the inclusion compounds of 1-MCP/α-CD or 1-MMCPCP/Cu-β-CD at six different concentrations obtained higher values than that of control fruits. The optimal effect was gained by the inclusion compounds of 1-MMCPCP/Cu-β-CD at 3200 nl/l. A similar effect on protein levels was also noted in fruits treated with the inclusion compounds of 1-MCP/α-CD at

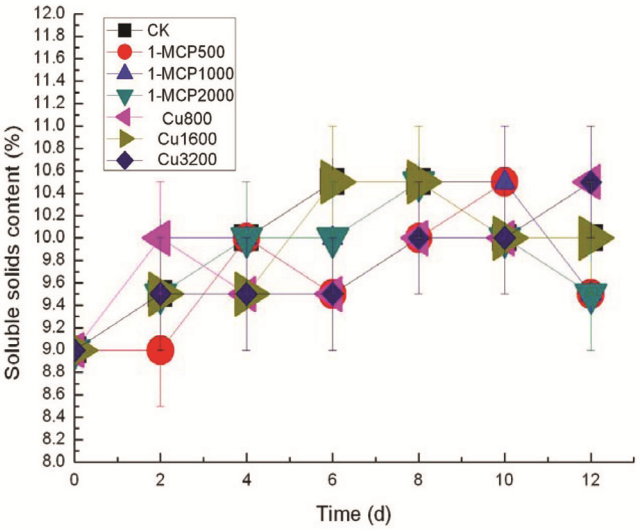

Figure 2. Effect of inclusion compounds of 1-MCP/α-CD or 1-MMCPCP/Cu-β-CD on the soluble solids content of sweet cherry.

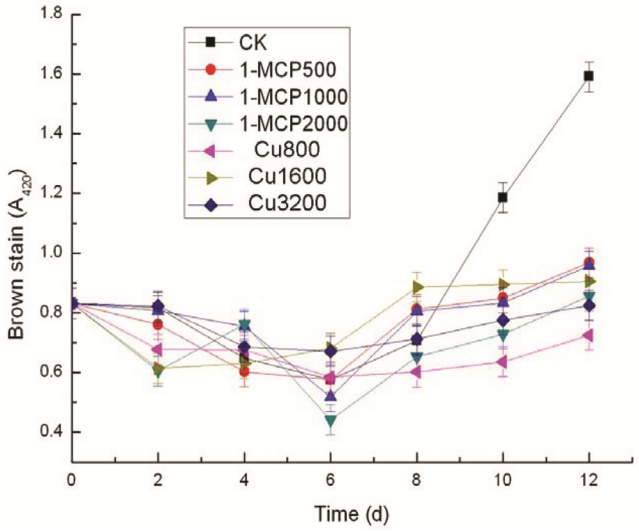

Figure 1. Effect of inclusion compounds of 1-MCP/α-CD and 1-MMCPCP/Cu-β-CD on the brown stain of sweet cherry.

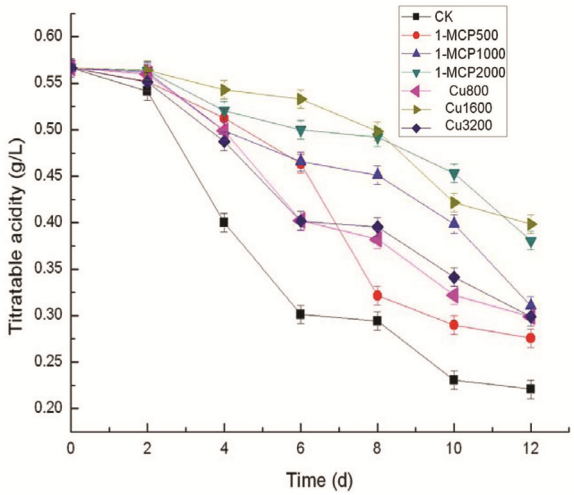

Figure 3. Effect of inclusion compounds of 1-MCP/α-CD or 1-MMCPCP/Cu-β-CD on the titratable acidity of sweet cherry.

a concentration of 2000 nl/l. In general, postharvest treatments of inclusion compounds of 1-MCP/α-CD or 1-MMCPCP/Cu-β-CD significantly maintained fruit protein levels compared to control fruits. 1-MCP prevented an increase in extraneous ethylene-induced electrolyte leakage, decrease in membrane protein, and decrease in liquid mobility in Petunia flowers[44]. In a non-ethylene environment, 1-MCP increased the longevity, fresh weight and total protein content of individual flowers compared to untreated controls, but had no effect on electrolyte leakage, membrane protein or liquid fluidity[45].

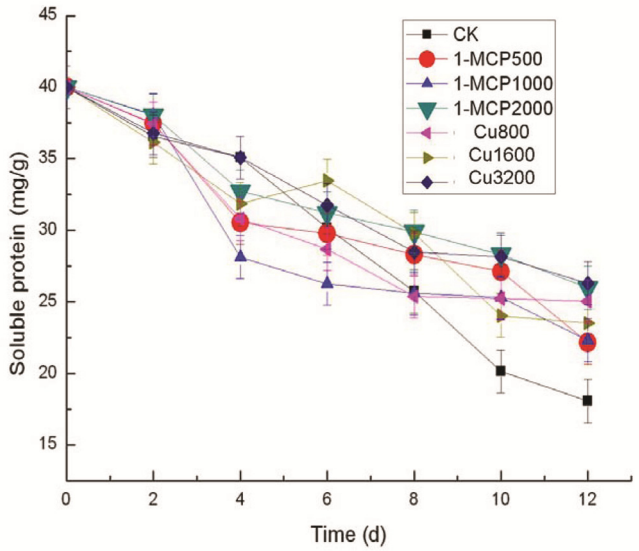

Figure 4. Effects of inclusion compounds of 1-MCP/α-CD or 1-MMCPCP/Cu-β-CD on the soluble protein content of sweet cherry.

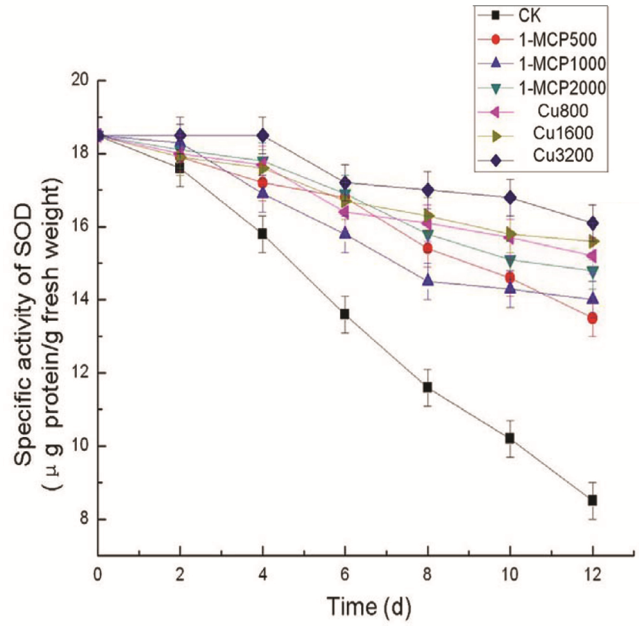

Figure 5. Effect of inclusion compounds of 1-MCP/α-CD or 1-MMCPCP/Cu-β-CD on the superoxide dismutase activity of sweet cherry.

The level of SOD activity was highly variable in cherry fruits treated. In general, 1-MCP or 1-MMCPCP treatment slowed down SOD activity. During the initial analysis of four days after harvest storage, a 15% reduction in SOD activity was observed in the control cherries. The SOD activity also declined on treatment with the inclusion compounds of 1-MCP/α-CD or 1-MMCPCP/Cu-β-CD during further postharvest storage (Figure 5). However, postharvest treatment with the inclusion compounds of 1-MCP/α-CD or 1-MMCPCP/Cu-β-CD brought out dramatic decrease in retarded SOD activity compared to controls. As shown in Figure 5, the SOD content of cherries treated with the inclusion compounds of 1-MCP/α-CD at 500 nl/l reduced most quickly among the six groups. Nonetheless, after 12 days, cherries treated with the inclusion compounds of 1-MCP/α-CD at 500 nl/l showed higher SOD activity value, viz. 13.5 (μg protein/g fresh wt) compared to the control fruits with 8.5 (μg protein/g fresh wt). Interestingly, SOD activity maintained nearly a fixed value in cherries treated with the inclusion compounds of 1-MMCPCP/Cu-β-CD at 3200 nl/l and reached nearly two-fold higher levels compared to the control fruits at day 12. An effective antioxidant system is essential for maintaining cell compartmentalization and preservation of nutrients and antioxidants[46–49]. As the first enzyme of the antioxidant system, SOD plays an important role in scavenging reactive oxygen species. During the 12-day storage period, SOD activity decreased with either the control fruit or fruits treated with the inclusion compounds of 1-MCP/α-CD or 1-MMCPCP/Cu-β-CD. However, SOD activity of the treated fruits declined more slowly than that of the control fruits. Also, fruits treated with the inclusion compounds of 1-MMCPCP/Cu-β-CD gained the slower decrease of SOD

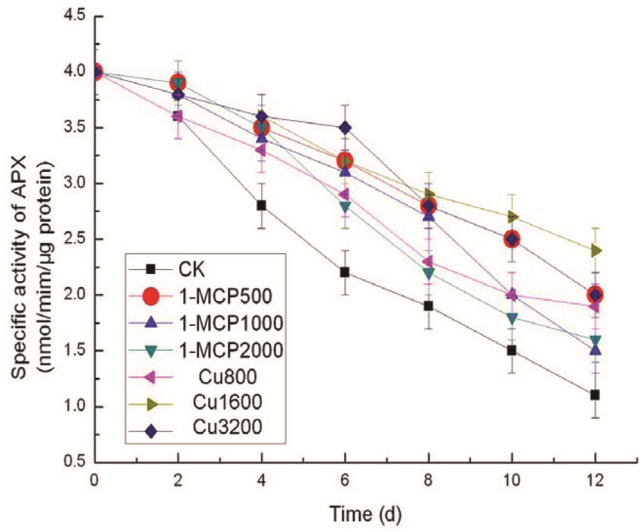

Figure 6. Effect of inclusion compounds of 1-MCP/α-CD or 1-MMCPCP/Cu-β-CD on the ascorbate peroxidase activity of sweet cherry.

activity as those of the inclusion compounds of 1-MCP/α-CD. A higher SOD activity in 1-MCP-treated fruits has been reported previously[50]. In addition, 1-MCP treatment enhanced the antioxidant capacity of fruits, resulting in high reactive oxygen species (ROS) scavenging potential[51]. Therefore, an increase in the antioxidant capacity in response to 1-MCP or 1-MMCPCP can provide beneficial effects in the preservation of sweet fruits.

There was no significant difference in the initial activity levels of APX (two days postharvest) between the control and treated cherries. In addition, although APX activity in control and different treatments decreased during further storage (Figure 6), it decreased in six differently treated sweet cherries after 12 days of storage compared to air-exposed fruits. Postharvest treatment of the inclusion compounds of 1-MCP/α-CD or 1-MMCPCP/Cu-β-CD showed significantly higher APX activity than that of the postharvest, air-exposed control fruits. With the concentration of 1600 nl/l for the inclusion compounds of 1-MMCPCP/Cu-β-CD, the reducing rate was the slowest, being 2.5 nmol/min/µg protein, which was two-fold higher in comparison to that of the air-stored fruits at day 12. However, pears treated with 1-MCP enhanced the activity of SOD, ascorbate peroxidase (POX), APX and catalase antioxidant enzymes, and reduced the incidence of core browning[51]. In addition, the activity of downstream enzymes such as ascorbate peroxidase is critical for the removal of hydrogen peroxide produced by SOD activity[51].

The results demonstrate that 1-MCP or 1-MMCPCP have a regulating function for sweet cherry. Furthermore, both the inclusion compounds restrained the ripening process of sweet cherry. The increasing rate for the browning process was effectively slowed down, being at least 1.6-fold higher than that of the controls fruits at day 12. The decreasing rate for TA and soluble protein content were both 1.25-fold higher than that of controls at day 12. And the inclusion compounds inhibit SOD and APX activity too, finally prolong the shelf life of sweet cherry. However, there were no major differences in SSC between control cherries and those treated with the inclusion compounds of 1-MCP/α-CD or 1-MMCPCP/Cu-β-CD after postharvest treatments and during extended storage. Thus, these two inclusion compounds are important in the preservation of sweet cherry. Owing to the lower price of β-cyclodextrin, the inclusion compound of 1-MMCPCP/Cu-β-CD should be a promising candidate for commercial use in extending the shelf life of sweet cherry.

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