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ORIGINAL RESEARCH ARTICLE

The effects of different storage temperatures combined with heat treatment on cucumber's quality and physiological and biochemical indexes

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ABSTRACT

The effects of different storage temperatures (2, 4 and 8 ℃) and their corresponding optimal heat treatment conditions on the quality, physiological and biochemical indexes of Cucumber Fruits during storage were studied by using the quadratic regression orthogonal rotation combination design. The effects of different storage temperatures (2, 4 and 8 ℃) and their corresponding optimal heat treatment conditions on the chilling injury, hardness, weightlessness rate, polyphenol oxidase (PPO), catalase (CAT), peroxidase (POD), H_2O_2, super oxygen anion free radical (O^{2-}), ASA and GSH were determined. The results showed that heat treatment could inhibit chilling injury, while heat treatment combined with 4 ℃ low temperature storage could effectively inhibit the decline of fruit hardness and weight loss rate, delay the increase of peroxidase (POD) and polyphenol oxidase (PPO) activities, inhibit the increase of H_2O_2 and superoxide anion free radical O^{2-} and significantly inhibit the browning of cucumber, delay the decline of ascorbic acid and maintain the content of GSH, it was beneficial to adjust the balance of active oxygen system. The results showed that under the storage condition of 4 ℃, the hot water treatment condition of cucumber was 39.4 ℃ and 24.3 min, which could delay the senescence of cucumber fruit and better maintain the quality of cucumber fruit.

Keywords: Cucumber Fruit; Chilling Injury; Storage Temperature; Quality; Active Oxygen

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1. Introduction

Cucumber (*Cucumis sativus* Linn.) is a common fruit and vegetable, its own moisture content is more than 98%, and contains rich in nutrients component[1]. During the storage and transportation of cucumbers, nutrients are lost rapidly, and they are easy to age and decay. Appropriate storage temperature can slow down the decay rate of fruits and prevent the reduction of nutrients[2]. Therefore, it is very important to determine the suitable storage temperature of cucumber.

Heat treatment is a physical treatment method without pollution and chemical residues. It has a significant effect on delaying the ripening and senescence of fruits and vegetables, reducing cold injury and inhibiting the activity of related enzymes[3]. Huan *et al.*[4] found that hot water treatment of peach fruit and storage at 4 ℃ had a significant effect on reducing chilling injury of peach and maintaining its quality. Zhang *et al.*[5] studied that after intermittent hot water treatment,

cucumber fruit stored at 10 ℃ and 40 ℃ for 30 or 50 min had the lowest decay and weight loss rate and the highest catalase activity. Nasef[6] found that the cucumber fruit treated with 55 ℃ hot water for 5 minutes, under the storage temperature of 4 ℃, has the possibility to maintain the sensory quality of cucumber, prolong the storage period, prevent decay and induce antioxidant enzymes. The results of heat treatment on pear[7], kiwi[8], loquat[9] and so on also show that it can improve the cold resistance.

Storage temperature is an important factor affecting the storage quality and shelf life of fruits and vegetables, and also an important factor affecting the metabolic process, quality and storage life of fruits[10]. Low temperature is conducive to inhibiting the physiological and biochemical reactions of post-harvest vegetables and delaying fruit aging[11], but inappropriate low temperature will cause abnormal metabolism of fruit and vegetable products. Qiao *et al*.[12] found that 0 ℃ can effectively delay the decline of nutrient content and the change of antioxidant enzyme activity in *Brassica rapa* L., significantly inhibit the post-harvest aging of *Brassica rapa* L., and effectively maintain the best edible quality and commercial characteristics of *Brassica rapa* L. Zhao *et al*.[13] studied that low temperature storage at 0 ℃ and 5 ℃ is conducive to maintaining the hardness of Gala apple, inhibiting the occurrence of decay, delaying aging, and better maintaining the fresh food quality of the fruit.

Previous studies were aimed at a specific storage temperature to study the effects of heat treatment on the physiological and biochemical characteristics of cucumber fruit, but there were few literatures on comparing different storage temperatures combined with heat treatment to study the storage characteristics of cucumber fruit. Therefore, this experiment took cucumber fruit as the test material, and used the optimal heat treatment conditions corresponding to different storage temperatures (2, 4 and 8 ℃) measured by quadratic regression orthogonal rotation combination design to treat cucumber fruit, in order to explore the effects of different storage temperatures and heat treatment conditions on the quality and physiological and biochemical characteristics of cucumber fruit, and provide a theoretical basis for the application of post-harvest cucumber in cold storage.

2. Materials and methods

2.1 Test materials and treatment

The cucumber variety selected in the experiment was “Shenqing” cucumber, which was transported back to the laboratory on the day of harvest after dispersing the field heat and removing the mechanical injury and pests. Sub packed in perforated polyethylene plastic bags, cucumber fruits with a mass of (260 ± 20) g and a length of (26 ± 2) cm were selected. There was no obvious mechanical damage on the surface of the fruits, and the fruits were allowed to stand at room temperature.

Thirty-six untreated cucumber fruits were randomly divided into three groups and stored in constant temperature and humidity boxes with temperature of 2, 4 and 8 ℃ and relative humidity of 85%–90%. They were divided into CK 2 ℃, CK 4 ℃ and CK 8 ℃ groups to measure the chilling injury index.

According to the previous combination design of conductivity and quadratic regression orthogonal rotation, the optimal heat shock temperature and time corresponding to different storage temperatures (2, 4 and 8 ℃) were determined: (1) under 2 ℃ storage temperature, the optimal heat treatment condition was heat shock in 38.7 ℃ hot water for 27.3 min (HWT 2 ℃); (2) under the storage temperature of 4 ℃, the best heat treatment condition was heat shock in 39.4 ℃ hot water for 24.4 min (HWT 4 ℃); (3) under the storage temperature of 8 ℃, the best heat treatment condition was heat shock in 40.6 ℃ hot water for 21.2 min (HWT 8 ℃). According to the results, the cucumber fruits were randomly divided into three groups for corresponding treatment. The temperature was adjusted through the constant temperature water temperature box, with 120 pieces in each group. After heat treatment, they were dried, put into 0.07 mm polyethylene film plastic bags respectively, and stored in constant temperature and humidity boxes with the temperature of 2, 4 and 8 ℃, and the relative humidity of 85%–90%, that is, HWT 2 ℃, HWT 4 ℃ and HWT 8 ℃. The chilling injury, weight loss rate,

hardness, catalase (CAT), peroxidase (POD), polyphenol oxidase (PPO), soluble solids, soluble protein, H_2O_2, superoxide anion free radical O^{2-}, ascorbic acid and reduced glutathione were measured once every 3 days. 24 pieces were taken from each group every time and repeated for 3 times.

1.2 Reagents and instruments

Reagents: thiobarbituric acid, trichloroacetic acid, absolute ethanol, sodium hydrogen phosphate, H_2O_2 kit, superoxide anion radical kit, sodium hydroxide solution, standard protein solution, ethanol solution, phosphoric acid, sodium hypochlorite (NaClO), potassium iodide, ascorbic acid, sulfuric acid, starch, glacial acetic acid, oxalic acid, sodium chloride, sodium bicarbonate, sodium thiosulfate, ETAS and hydrogen peroxide, all of which are analytical pure.

Instrument: GY-4 digital display hardness tester, Zhejiang Topuyunnong Technology Co., Ltd; BPS-100CA constant temperature and humidity incubator, Shanghai Santeng Instrument Co., Ltd; H-2050R-1 high speed refrigerated centrifuge, Shandong Boke Scientific Instruments Co., Ltd; UV-Visible spectrophotometer, Shanghai Meipuda Instrument Co., Ltd; HSWX-600BS electric thermostatic water temperature box, Shanghai Huyueming Scientific Instrument Co., Ltd; THZ-82A constant temperature oscillation box, Changzhou Jintanyoulian Instrument Research Institute; BJ2100D digital hole electronic balance, Shanghai Yifen Scientific Instrument Co., Ltd.

1.3 Method

1.3.1 Determination of chilling injury index

The chilling injury index of cucumber fruit was evaluated according to the method of Cao *et al*.[14] When twelve cucumbers were randomly selected, the chilling injury index was divided into five grades: grade 0, without chilling injury; grade 1, the chilling damage area shall not exceed 25%; grade 2, mild chilling injury, with chilling injury area accounting for 25%–50%; grade 3, moderate chilling injury, with chilling injury area accounting for 50%–75%; grade 4, severe chilling injury, the chilling injury area exceeds 75%. Calculate the chilling injury index according to formula (1) and repeat the measurement for 3 times.

$$\text{Chilling injury index} = \frac{\Sigma(Chilling\ injury\ index + Number\ of\ fruits\ in\ this\ grade}{4 \times The\ total\ number\ of\ fruits\ was\ determined} \quad (1)$$

The fruits were taken out on the 3rd, 6th, 9th, 12th and 15th days respectively, and placed at room temperature for 3 days to observe the chilling injury of fruits in each group.

1.3.2 Determination of weight loss rate and hardness

The weight loss rate is measured by weighing method[15] and repeated for 3 times for each treatment.

$$\text{Weight loss rate} = \frac{m_0 - m_1}{m_1} \times 100\% \quad (2)$$

Where: m_0 is the original mass of cucumber fruit, g; m_1 is the mass of cucumber fruit after storage, g.

The hardness shall be measured by GY-4 digital display hardness tester. At the equatorial part of cucumber fruit, take 1 cm thick pulp, and use a durometer to vertically drive it into the cucumber pulp for measurement. The results are expressed in N and repeated for 3 times.

1.3.3 Determination of soluble solids and soluble proteins

The content of soluble solids was determined by Abbe refractometer with reference to the method of Li *et al*.[16], and repeated for 3 times in each treatment group. The soluble protein was determined by the ultraviolet absorption method of Cao *et al*.[14], 1 g of the sample was weighed into 3 mL of distilled water, and the absorbance at 280 nm was measured.

1.3.4 Determination of CAT, POD and PPO activities

Referring to the method of Li[17], the content of

pod was determined by guaiacol method. Add 3.0 mL of 25 mmol·L^{-1} guaiacol and 0.5 mL of enzyme extract respectively, and then add 0.25 mL of H_2O_2 solution. The reaction was quickly started to determine the absorbance value at 470 nm. For the determination of polyphenol oxidase activity, refer to the method of Jayachandran *et al*.[18]: the product formed after PPO catalysis has the maximum light absorption peak at 420 nm, which can be determined by colorimetry, expressed in U·g^{-1}, and repeated for 3 times. The determination of cat is slightly modified according to the method of Chen *et al*.[19], and the activity of the enzyme can be determined according to the change of H_2O_2 Content during the reaction. The change of H_2O_2 content can be detected by UV spectrophotometer, and the change of OD_{240} value within 1 min per gram of pulp is taken as an enzyme activity unit.

1.3.5 Determination of H_2O_2 content and superoxide anion radical (O^{2-})

H_2O_2 content is measured by H_2O_2 test box[20]. According to the kit, determine the sample quantity before the test to prepare tissue homogenate; determine according to the kit method, and the content is expressed in μmol·g^{-1}. Repeat the determination for 3 times. The production rate of O^{2-} was determined by using the superoxide anion free radical test box: determine the sampling amount, take 1g of cucumber pulp, add 4ml of phosphate buffer with pH value of 7.2, grind it in ice bath, centrifuge it at 4 ℃ and 8,000 r·min^{-1} for 20 min, and determine it according to the method of the kit, the unit is U·g^{-1}, repeat the determination for 3 times.

1.3.6 Determination of ascorbic acid (ASA) content and reduced glutathione (GSH)

The ASA content was determined by 2, 6-dichloroindophenol method with reference to the method of Wang *et al*[21]. Take (5 ± 0.1) g cucumber fruit sample, add 0.2 mol·L^{-1} oxalic acid to homogenize it, and then fix the volume. Take 20 mL of the filtrate for titration until the dark blue of 2, 6-dichloroindophenol solution is reduced to colorless. The determination was repeated 3 times for each treatment.

The determination of GSH refers to the method of Cao *et al*.[14] The substance has the maximum light absorption at the wavelength of 412 nm, so the content of GSH in fruit can be determined by spectrophotometry. Take 3.0 g cucumber tissue sample, add 5 mL of 50 g·L^{-1} trichloroacetic acid solution for ice bath grinding, centrifuge at 4 ℃ for 20 min at 8,000 r·min^{-1}, and then measure its absorbance at 412 weight loss rate nm. The results are as follows: μmol·g^{-1} indicates that the determination is repeated 3 times for each treatment.

1.4 Data analysis

The data were processed by Excel 2010 software, analyzed by SPSS21.0 software and compared by Duncan.

2. Results and analysis

2.1 Effect of different storage temperature combined with heat treatment on chilling injury index of cucumber fruit

Chilling injury index is one of the important indexes to reflect the quality of cucumber fruit. As shown in **Figure 1**, the chilling injury index of cucumber fruits under different storage temperatures showed an upward trend with the extension of storage time. When cucumber fruits were stored for 3 days, chilling injury occurred in heat treatment group and CK group at 2, 4 ℃ storage temperature, and no chilling injury occurred in heat treatment group and CK group at 8 ℃; on the 6th day of storage, chilling injury occurred in the heat treatment group and CK 2, 4 and 8 ℃ groups at storage temperature, and with the extension of storage time, the chilling injury index at 2 and 4 ℃ in the heat treatment group and CK group was significantly higher than 8 ℃. The chilling injury of cucumber fruits in heat treatment group and CK group was aggravated at 2 ℃ and 4 ℃ storage temperature at the later stage of storage. On the 12th day of storage, the chilling injury indexes of cucumber fruits at HWT 2 ℃, HWT 4 ℃ and HWT 8 ℃ storage temperature were 0.69, 0.6 and 0.2 respectively. The chilling injury indexes of cucumber fruits stored at CK 2 ℃, CK 4 ℃ and CK 8 ℃ were 0.74, 0.63 and 0.23 respectively. The results showed that heat treatment could significantly reduce the

chilling injury index of cucumber fruit. The chilling injury index of cucumber fruit in heat treatment group and CK group was lower at 8 ℃ storage temperature, indicating that the chilling injury index was kept low at 8 ℃. However, at 8 ℃ storage temperature, the tail of cucumber fruit shrank seriously after storage, which may be due to the increase of storage temperature, which led to the loss of water and the decline of the marketability of cucumber fruit.

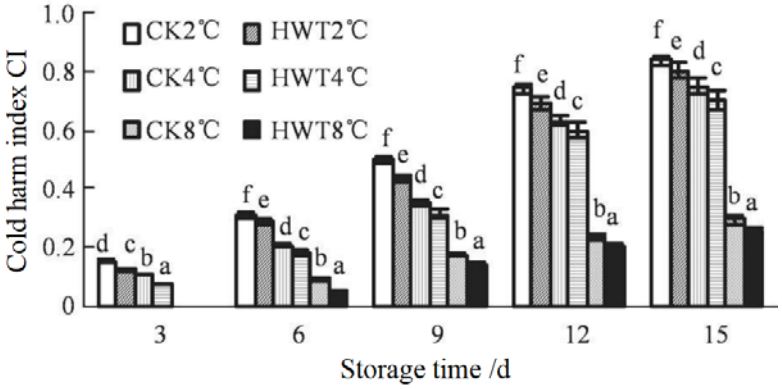

Figure 1. Effects of different storage temperature combined with heat treatment on CI of cucumber fruits.

2.2 Effects of different storage temperatures combined with heat treatment on weight loss rate and hardness of cucumber fruit

Storage temperature is an important factor affecting the storage quality of cucumber. The higher the temperature, the more vigorous the respiration. Therefore, suitable low-temperature storage can inhibit water transpiration and effectively maintain the commodity value. It can be seen from **Figure 2** that during the whole storage period, the weight loss rate of cucumber fruits at different storage temperatures showed an upward trend with the increase of storage time, and the weight loss rate of HWT 8 ℃ was significantly higher than that of other storage temperatures ($P < 0.05$).

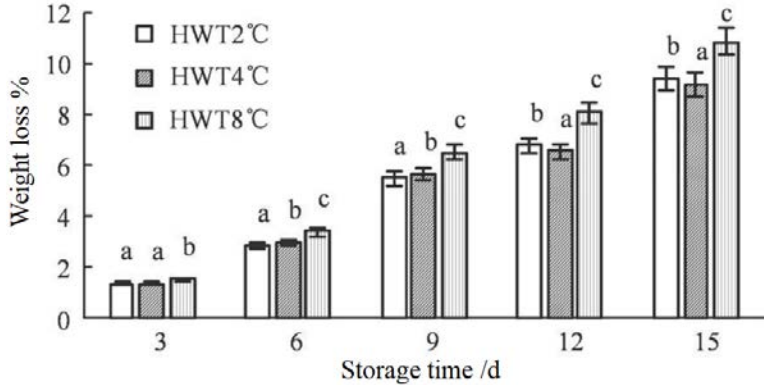

Figure 2. Effects of different storage temperature combined with heat treatment on weight loss rate of cucumber fruits.

With the prolongation of storage time, HWT 8 ℃ cucumber peel appeared wrinkles and severe water loss. The results showed that in the early stage of storage, the lower the storage temperature was, the smaller the increase rate of weight loss of cucumber fruit was, which was conducive to the water conservation of cucumber during storage. HWT 2 ℃ storage could inhibit the increase of weight loss rate and delay the loss of cucumber fruit quality. It shows that low temperature can effectively inhibit the transpiration of cucumber and reduce the evaporation of water, which is consistent with the research results of Hunan local green pepper[22] and green bamboo shoots[23]. It can be seen that temperature is an important factor affecting the weight loss rate and hardness during postharvest storage. Low temperature can maintain low weight loss rate and high hardness during storage, so as to

maintain storage quality and prolong shelf life. At the later stage of storage, with the aggravation of chilling injury, the weight loss rate of HWT 2 ℃ storage was gradually higher than that of HWT 4 ℃, indicating that the degree of chilling injury deepened, resulting in the aggravation of water loss of cucumber fruit.

Cucumber fruit hardness is one of the important indexes to measure its storage life. During storage, due to the action of enzymes, cell wall substances are decomposed, resulting in fruit softening[24]. As shown in **Figure 3**, the changes of cucumber fruit hardness under different storage temperatures combined with heat treatment showed a downward trend. With the prolongation of storage time, the storage condition of HWT at 8 ℃ decreased obviously. There was no significant difference in fruit hardness between HWT 2 ℃ and HWT 4 ℃ 3 days before storage ($P < 0.05$). At the later stage of storage, the fruit hardness of cucumber at HWT 4 ℃ decreased slowly compared with that at HWT 2 ℃ and HWT 8 ℃. After 15 days of storage, the decreases of HWT 2 ℃, HWT 4 ℃ and HWT 8 ℃ were 21.58%, 20.48% and 25.3% respectively. The results showed that the appropriate storage temperature had a significant effect on inhibiting the decline of cucumber fruit hardness. In the early stage of storage, the increase of storage temperature will accelerate the softening of cucumber fruit, and the decrease of hardness is related to the loss of water. Under the condition of heat shock, the 8 ℃ storage temperature causes the hardness because of the serious loss of water dispersion, while HWT 2 ℃ is helpful to inhibit the decline of hardness; with the aggravation of chilling injury at the later stage of storage, the decline rate of flesh hardness at HWT 2 ℃ was faster than that at HWT 4 ℃. The results showed that the degree of chilling injury affected the decrease of hardness. The reason for the decrease may be that with the deepening of chilling injury, the decomposition of cell wall substances led to the deepening of cell softening.

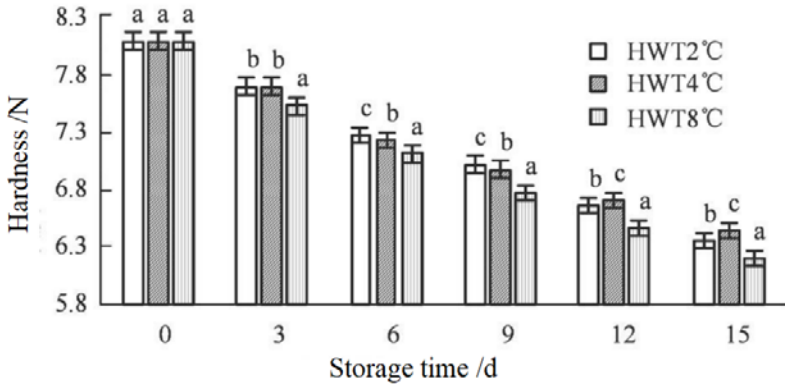

Figure 3. Effects of different storage temperature combined with heat treatment on hardness of cucumber fruits.

2.3 Effects of different storage temperatures combined with heat treatment on the contents of soluble solids and soluble proteins in cucumber fruits

Soluble solids can reflect the quality and nutritional value of fruit, and have an important impact on the taste and storage of fruit[25], which is one of the important indicators to evaluate fruit quality. It can be seen from **Figure 4** that the content of soluble solids increased first and then decreased at different storage temperatures. On the third day, the content of soluble solids increased slightly and then decreased gradually. On the third day of storage, the highest increase rate of cucumber fruit at 4 ℃ of HWT was due to the hydrolysis of starch under the action of hydrolase, which increased the soluble solids. With the extension of storage time, the soluble solids content gradually decreased. During storage, there was little difference in soluble solid content between HWT 2 ℃ and HWT 4 ℃. After 6 days storage, the soluble solid content of HWT at 8 ℃ decreased the most, and with the higher storage temperature, the faster the consumption of soluble solids. It showed that low temperature could effectively delay the consumption of soluble solids. At the later stage of storage, the degree of chilling injury of cucumber fruits stored at HWT 2 ℃ deepened, which enhanced the permeability of cell

membrane and aggravated the consumption of organic matter. The results showed that the accumulation of sugar in the early stage of storage led to the increase of soluble solids, and the decrease of soluble solids in low temperature storage was slow, which was consistent with the test results of passion fruit[26] and *Litchi* fruits[27].

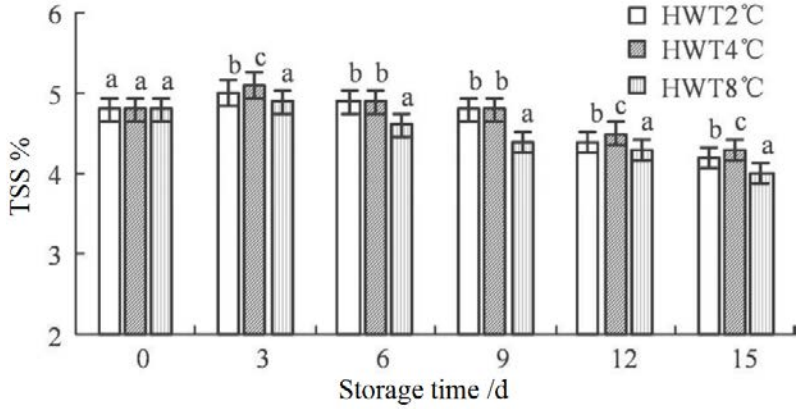

Figure 4. Effects of different storage temperature combined with heat treatment on TSS of cucumber fruits.

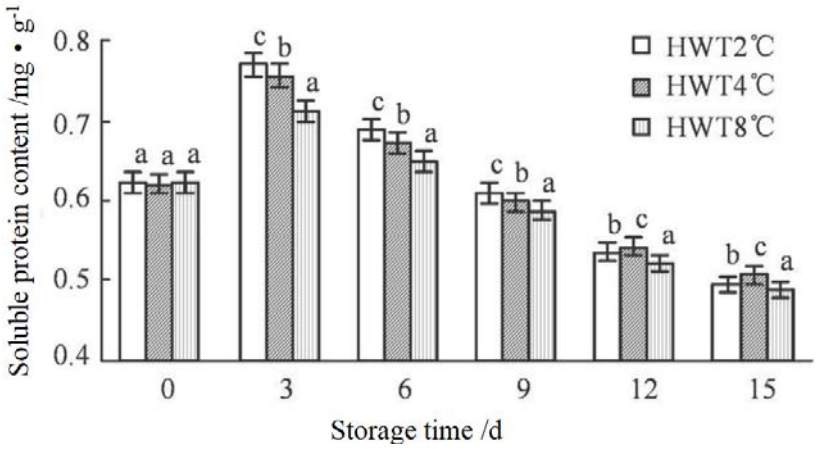

Figure 5. Effects of different storage temperature combined with heat treatment on soluble protein in cucumber fruits.

The content of soluble solids in cucumber fruits stored at HWT 4 ℃ could be well maintained, and the quality of Cucumber Fruits during storage could be well maintained. The accumulation of soluble proteins can improve the osmotic regulation of plants and better maintain the integrity of cell membrane[28]. It can be seen from **Figure 5** that the soluble protein content of Cucumber Fruit under different storage temperatures combined with heat treatment increases rapidly and then decreases slowly with the change of storage time. During storage, the content of soluble protein showed significant difference at different storage temperatures ($P < 0.05$). On the third day of storage, the soluble protein content at different storage temperatures reached the peak. The soluble protein content at HWT 2 ℃ increased the fastest, 24.2%, and the soluble protein content at HWT 4 ℃ and 8 ℃ increased by 18.4% and 14.3%. At the later stage of storage, the soluble solid protein content gradually decreased, and there were significant differences among the treatment groups ($P < 0.05$). The soluble protein content of cucumber fruit in HWT 2 ℃ group decreased the fastest. At the end of storage, the soluble protein content of HWT 4 ℃ was higher than that of HWT 2 ℃ and HWT 8 ℃. On the 15th day, the soluble protein content of HWT 4 ℃ was 3.46% higher than that of HWT 8 ℃. The results showed that in the early stage of storage, the content of soluble protein increased under low temperature stress, and the content of HWT increased fastest at 2 ℃; at the later stage of storage, with the extension of storage time, the higher the storage temperature is, the more soluble protein is consumed. With the deepening of chilling injury, the loss of soluble protein content at 2 ℃ storage tem-

perature of HWT is aggravated. It showed that low temperature significantly inhibited the decline of soluble protein content, and HWT 4 ℃ storage could better maintain cell viability, which was conducive to maintaining its nutritional quality.

2.4 Effects of different storage temperatures combined with heat treatment on the activities of CAT, POD and PPO in cucumber fruits

In the membrane lipid peroxidation reaction, catalase plays an important role in plant growth and development, anti-aging, etc., which can reduce the adverse effects on the body caused by reactive oxygen species[29], effectively remove free radicals and prevent the damage of reactive oxygen species to plants[30].

As shown in **Figure 6**, the CAT enzyme activity showed a trend of increasing first and then decreasing. On the 9th day of storage, the CAT activity of each treatment group reached the peak, and then decreased gradually. At 9 days before storage, the CAT activity of cucumber fruits at HWT 4 ℃ was significantly higher than that at other storage temperatures. The CAT activities at HWT 2 ℃, HWT 4 ℃ and HWT 8 ℃ were 25.9, 28.64 and 24.8 $U \cdot g^{-1}$, respectively, indicating that CAT activity in fruits was induced to increase and scavenge reactive oxygen species at appropriate storage temperatures. At the later stage of storage, with the deepening of chilling injury, the CAT activity of cucumber fruits at 8 ℃ of HWT decreased slowly. At 15 days' storage, the CAT activities of HWT at 2 ℃, 4 ℃ and 8 ℃ were 14.32, 17.12 and 15.48 $U \cdot g^{-1}$ respectively, indicating that the CAT activity of HWT at 8 ℃ was higher than that of HWT at 2 ℃ due to the deepening of chilling injury in the later stage of storage, which reduced the damage degree of active oxygen substances. The results showed that the CAT activity of cucumber fruit at 4 ℃ of HWT had a significant effect on the removal of H_2O_2 accumulated in plants.

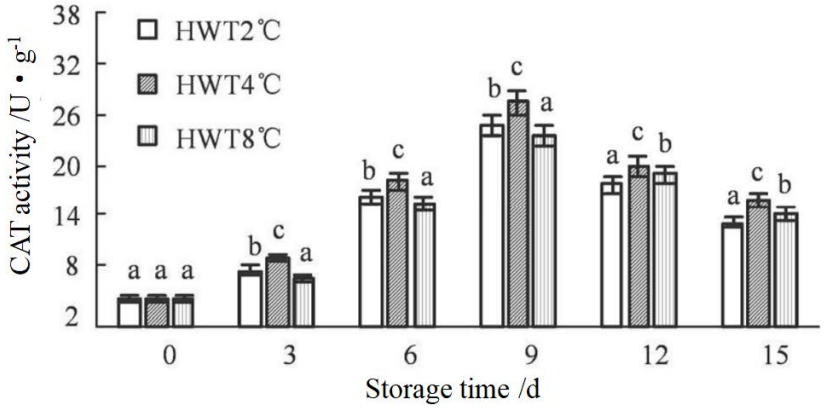

Figure 6. Effects of different storage temperature combined with heat treatment on CAT in cucumber fruits.

Peroxidase (POD) is an important protective enzyme in the enzymatic defense system to prevent the peroxidation of plant membrane lipids. It can catalyze the cracking of hydroperoxides of unsaturated fatty acids, reduce the damage of H_2O_2 to cells, and produce free radicals[31], which plays an important role in the development of fruit senescence and chilling injury, and is also an important indicator of fruit senescence[32]. It can be seen from **Figure 7** that POD activity showed an upward trend and increased with the extension of storage time. There were significant differences in POD activity among different treatment groups ($P < 0.05$). The POD activity of cucumber fruits stored at 4 ℃ was lower than 2 ℃ and 8 ℃ 6 days before storage; on the 6th day of storage, the treatment groups of HWT 2 ℃, HWT 4 ℃ and HWT 8 ℃ were 2.03, 1.91 and 2.66 times higher than that of 0 day, respectively, and the inhibition effect of HWT 4 ℃ was the most obvious; after 9 days storage, the POD activity of HWT 2 ℃ was lower than that of HWT 4 ℃ and HWT 8 ℃; at the end of storage, the POD activity of cucumber fruits stored at HWT 8 ℃ increased from 3.38 $U \cdot g^{-1}$ at 0 d to 17.26 $U \cdot g^{-1}$ at 15 days. It indicates that low temperature can reduce the activity of catalase and the production of free

radicals, which may be due to the increase of free radicals, inducing the synthesis of POD and increasing the activity of POD.

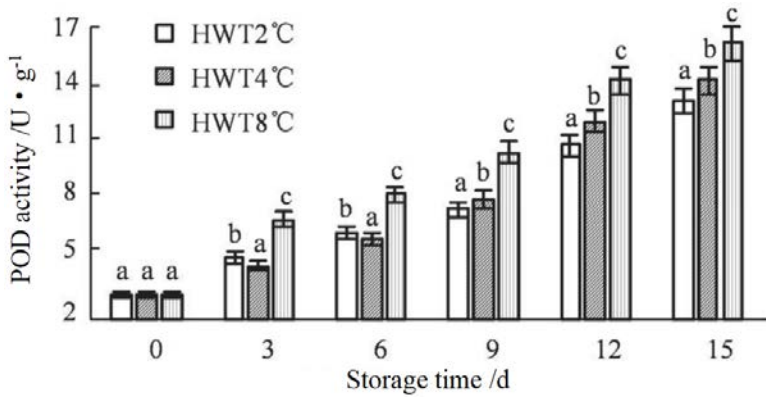

Figure 7. Effects of different storage temperature combined with heat treatment on POD in cucumber fruits.

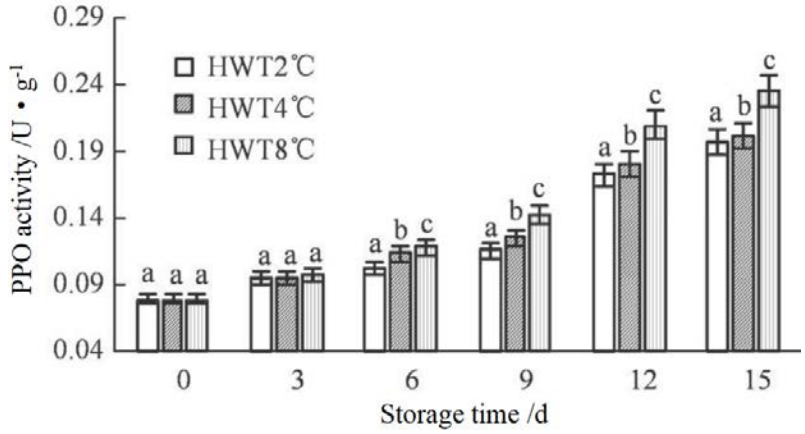

Figure 8. Effects of different storage temperature combined with heat treatment on PPO in cucumber fruits.

Polyphenol oxidase (PPO) can catalyze the oxidation of a variety of phenolic substances to quinone compounds[33]. These compounds further polymerize to form brown. The level of PPO activity can directly affect the degree of fruit browning and occurrence. It can be seen from **Figure 8** that PPO enzyme activity increases gradually with the extension of storage time, and PPO enzyme activity increases rapidly at 8 ℃ storage temperature of HWT, while PPO enzyme activity increases slowly at 2 ℃ storage temperature of HWT. After 15 days storage, PPO enzyme activity reached 0.197, 0.202 and 0.236 U·g^{-1}, respectively, with significant difference ($P < 0.05$), indicating that low temperature could significantly reduce polyphenol oxidase activity, inhibit membrane lipid peroxidation and delay browning.

Gu *et al.*[34] explored the effects of storage temperature (0, 5 and 10 ℃) on postharvest physiological quality and antioxidant enzyme activity of soft jujube kiwifruit, and found that 0 ℃ storage significantly inhibited the browning of soft jujube kiwifruit, maintained high fruit hardness, inhibited the increase of POD and PPO activities, and maintained high antioxidant enzyme activity, which is consistent with the research results of this experiment. This study found that pod and PPO were positively correlated. Compared with HWT 8 ℃, the pod and PPO activities of fruits stored at HWT 2 ℃ and HWT 4 ℃ were lower, which may be because low temperature stress inhibited cell respiration and biological metabolism, and improved antioxidant enzyme activity and membrane stability.

2.5 Effects of different storage temperatures combined with heat treatment on hydrogen peroxide content (H_2O_2) and superoxide anion radical (O^{2-}) in cucumber fruit

Hydrogen peroxide (H_2O_2) is an active oxygen free radical in plants, and it is the initiating factor

causing aging. When the metabolism of active oxygen scavenging system is unbalanced, H_2O_2 will accumulate in large quantities, resulting in the destruction of cell membrane structure. It can be seen from **Figure 9** that during storage, the H_2O_2 Content of cucumber fruits in each treatment group showed an overall upward trend, and there was a significant difference between the H_2O_2 Content of cucumber fruits at HWT 4 ℃ and HWT 2 ℃ and HWT 8 ℃ ($P < 0.05$).

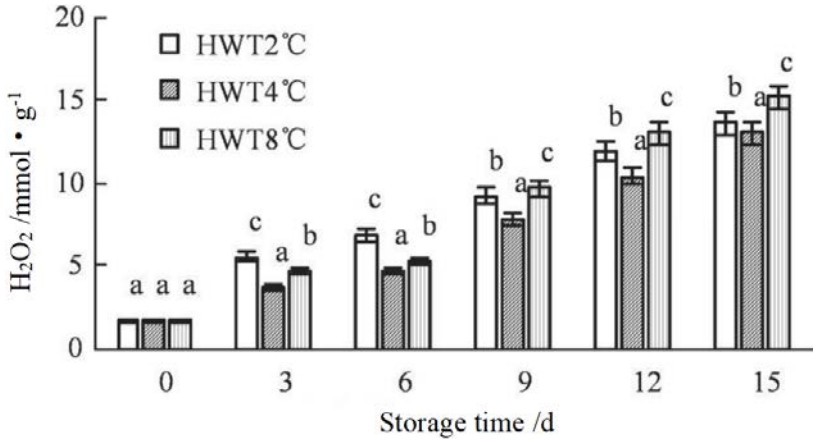

Figure 9. Effects of different storage temperature combined with heat treatment on H_2O_2 Content in cucumber fruits.

Six days before storage, the H_2O_2 Content of HWT 2 ℃ group was significantly higher than that of HWT 4 ℃ and HWT 8 ℃, which may be related to the deepening of chilling injury in the early stage of storage, resulting in the deepening of cell membrane damage and the accumulation of H_2O_2 content; on the 6th day of storage, the H_2O_2 Content of HWT 2 ℃, HWT 4 ℃ and HWT 8 ℃ was 4, 2.74 and 3.09 times higher than that of 0 day, respectively; at the later stage of storage, the H_2O_2 Content of HWT 8 ℃ was significantly higher than that of HWT 2 ℃ and HWT 4 ℃; on the 15th day of storage, HWT 8 ℃ was 9.8% and 13.8% higher than HWT 2 ℃ and HWT 4 ℃, respectively. The results showed that HWT 4 ℃ group could significantly inhibit the increase of H_2O_2 Content in cucumber fruit, which was beneficial to reduce H_2O_2 in fruit, slow down its damage and delay fruit senescence.

Lipid peroxidation can lead to disease and tissue damage. During the ripening process of fruit, a large amount of reactive oxygen species will be accumulated, which has strong oxidation ability and accelerates the internal oxidative damage of fruit. With the continuous accumulation of O^{2-} the integrity of cells will be destroyed, and the membrane structure and function will be damaged. However, the body will also defend against this reaction, so that reactive oxygen species can be maintained at a low level to prevent its toxic effect. It can be seen from **Figure 10** that with the extension of storage time, the content of superoxide anion free radical under different storage temperatures showed an upward trend. In the early stage of storage, the O^{2-} stored in HWT 2 ℃ group was significantly higher than that in HWT 4 ℃ and HWT 8 ℃ groups ($P < 0.05$). On the third day of storage, the storage of HWT2, HWT4 and HWT 8 ℃ groups were 1.85, 1.65 and 1.77 times higher than that of 0 days, respectively, indicating that the storage of HWT 4 ℃ group inhibited the increase of O^{2-} content in cucumber fruits, improved the antioxidant activity of fruits, and better maintained the quality of fruits; at the later stage of storage, the content of superoxide anion free radical at HWT 8 ℃ was always higher than that at HWT 2 ℃ and HWT 4 ℃. At 15 days of storage, the content of superoxide anion free radical at HWT 2 ℃, HWT 4 ℃ and HWT 8 ℃ was 4.68, 4.45 and 5.36 times higher than that at 0 days, respectively. The increase of O^{2-} content in cucumber fruits stored at HWT 8 ℃ showed that their antioxidant activity gradually decreased and the fruits gradually aged.

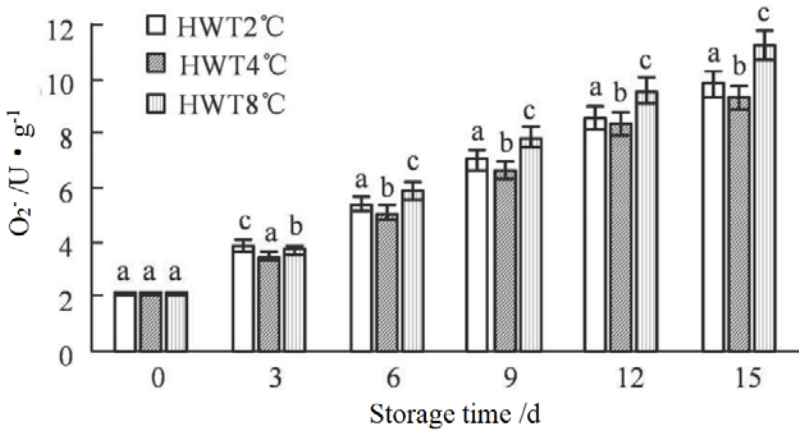

Figure 10. Effects of different storage temperature combined with heat treatment on $O^{2-}\cdot$in cucumber fruits.

2.6 Effects of different storage temperatures combined with heat treatment on ascorbic acid content (ASA) and reduced glutathione content (GSH) of cucumber fruit

Ascorbic acid (ASA) is an important indicator of the nutritional quality of fruits and vegetables. It can resist the damage of free radicals to cells, have antioxidant activity, and delay the aging of fruits and vegetables[35]. It can be seen from **Figure 11** that the ASA content of cucumber fruit tends to decrease with the extension of storage time, and the ASA content under the storage condition of HWT 4 ℃ is significantly higher than that under the storage conditions of HWT 2 ℃ and HWT 8 ℃, indicating that the ASA content can be better maintained under the low temperature storage with appropriate temperature. On the 6th day of storage, ASA content of cucumber decreased to 21.5%, 15.85% and 27.59% of that of 0 day at HWT 2 ℃, HWT 4 ℃ and HWT 8 ℃, respectively; on the 15th day of storage, ASA content of cucumber at HWT 2 ℃, HWT 4 ℃ and HWT 8 ℃ was 53.62%, 49.47% and 51.52% lower than that at 0 day, respectively. In the early stage of storage, ASA content decreased rapidly at 8 ℃ of HWT, indicating that the increase of temperature led to the decrease of ASA content in cucumber fruit; at the later stage of storage, ASA content decreased significantly at 2 ℃ storage temperature of HWT, which was due to the serious damage of cell membrane with the deepening of chilling injury, resulting in the loss of nutrients. Under HWT 4 ℃, ASA content of cucumber fruit decreased slowly, which could maintain the quality of Cucumber Fruit during storage.

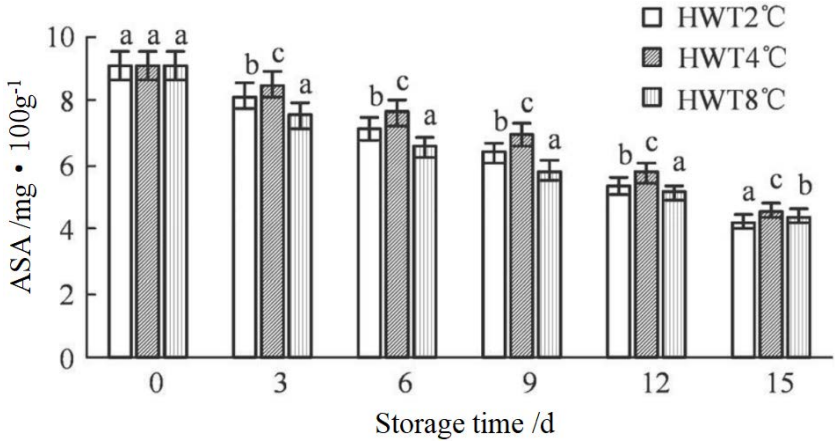

Figure 11. Effects of different storage temperature combined with heat treatment on ASA content in cucumber fruits.

Reduced glutathione (GSH) can be used as a hydrogen donor to convert H_2O_2 into H_2O, remove lipid hydroperoxides, reduce the damage of organic hydroperoxides to the body, inhibit lipid peroxidation induced by reactive oxygen species, and play an important role in preventing aging. ASA-GSH cycle is a combination of non-enzymatic antioxidants, which can play a role in scavenging reactive oxygen species and inhibiting membrane lipid peroxidation. It can be seen from **Figure 12** that the

GSH content in cucumber fruits at different storage temperatures showed a trend of slowly increasing at first and then decreasing, reaching the peak on the 12th day.

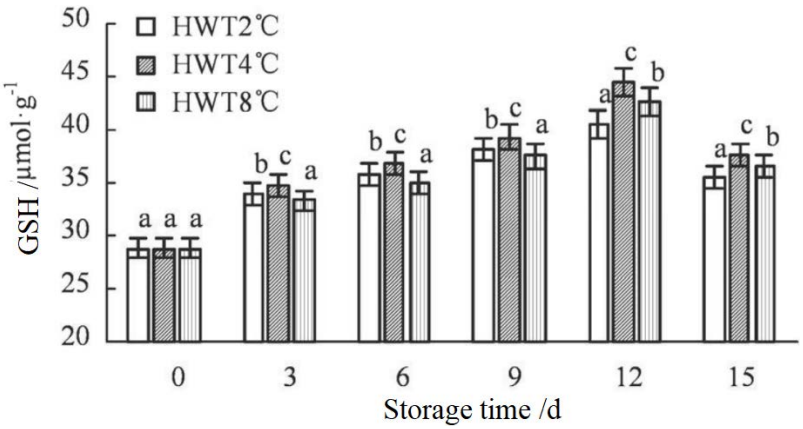

Figure 12. Effects of different storage temperature combined with heat treatment on GSH content in cucumber fruits.

After storage for 9 days, the GSH content of HWT 2 ℃, HWT 4 ℃ and HWT 8 ℃ increased from 28.68 μmol·g^{-1} at 0 days to 38.03, 39.06 and 37.41 μmol·g^{-1} respectively; at the end of storage, the GSH contents of HWT 2 ℃, HWT 4 ℃ and HWT 8 ℃ were 35.32, 37.54 and 36.42 μmol·g^{-1} respectively. The results showed that in the early stage of storage, HWT 2 ℃ and HWT 4 ℃ increased more than HWT 8 ℃; at the later stage of storage, with the deepening of chilling injury, the GSH content of HWT 2 ℃ group was lower than that of other storage temperatures. Under the storage temperature of HWT 4 ℃, the GSH content of cucumber fruit increased the most, which contributed to the accumulation of reactive oxygen species in cucumber fruit.

3. Conclusion

After heat treatment and low temperature storage, all life activities of cucumber will be inhibited. Low temperature will inhibit the activity of enzymes, and the rate of biochemical reaction of enzymes will slow down at low temperature, which can reduce the deterioration of cucumber quality. In this experiment, under different storage temperatures, heat treatment can significantly reduce the chilling injury index of cucumber fruit and inhibit the aggravation of chilling injury, which shows that heat treatment plays a positive role in the storage of cucumber fruit. According to the optimal heat treatment conditions corresponding to the storage temperature of 2 ℃, 4 ℃ and 8 ℃ determined by the quadratic regression orthogonal rotation combination design in the previous experiment, it was found that appropriate low temperature could inhibit the decline of weight loss rate and hardness, and maintain the metabolic balance of active oxygen scavenging system. The effect of low temperature storage of 2 ℃ and 4 ℃ in the heat treatment group on the weight loss rate and hardness of cucumber fruit was significantly better than that of 8 ℃ in the heat treatment group, the chilling injury index at 2 ℃ and 4 ℃ in the heat treatment group was more serious than that at 8 ℃, but at the later stage of storage, the tail of cucumber fruit in the heat treatment group had obvious water loss and shrinkage, which affected the marketability of cucumber fruit. Low temperature storage at HWT 2 ℃ and HWT 4 ℃ could effectively inhibit the deterioration of nutrients in cucumber fruit, the decrease of fruit hardness, soluble solids and ascorbic acid, the accumulation of H_2O_2 and O^{2-} and the damage of cell membrane structure, delay the decline of nutrients, and slow down the senescence and browning of Cucumber fruit. By comparison, under the storage condition of HWT 4 ℃ group (hot water treatment condition of 39.4 ℃ and 24.3 min), the storage effect of cucumber is better, which can better maintain the balance of nutrition and active oxygen metabolism. It is a more suitable storage condition for cucumber fruit.

Conflict of interest

The authors declared no conflict of interest.

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