Food Chemistry: X 23 (2024) 101599

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x

Influence of different pre-cooling methods on the freshness preservation of bok choi(*Brassica rapa* var. *chinensis*)

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ARTICLE INFO

Keywords:
Bok choi
Pre-cooling method
Vacuum pre-cooling
Postharvest quality
Antioxidant enzymes

ABSTRACTS

Several pre-cooling methods for bok choi were used, such as natural convection pre-cooling (NCPC), strong wind pre-cooling (SWPC), vacuum pre-cooling (VPC), cold water pre-cooling (CWPC), electrolyzed water pre-cooling (EWPC), and fluid ice pre-cooling (FIPC), in order to determine the most suitable precooling method. It was found that VPC reduced the respiration rate, inhibited the increase of malondialdehyde (MDA) and relative electrolyte leakage, and significantly decreased the total bacterial count. This may be due to the rapid decompression process during vacuum pre-cooling, which disrupts the microbial structure and has a certain sterilizing effect. Bok choi pre-cooled by VPC had the best color, hardness value, chlorophyll, titratable acid (TA) content, vitamin C (VC) content, total phenolic (TP) content, soluble sugar content, superoxide dismutase (SOD) activity, ascorbate peroxidase (APX) activity, and catalase (CAT) activity. Therefore, the most suitable pre-cooling method for bok choi among the above pre-cooling methods was the VPC method.

1. Introduction

Bok choi (*Brassica rapa* L. *ssp. chinensis*) belongs to *Brassica L.*, is rich in vitamins, sugar, acid, and other nutrients, and is a common leafy vegetable on the dining table. Bok choi is deeply loved by Chinese consumers with a huge annual production and demand. Similar to other leafy vegetables, bok choi has a large leaf surface area, high water content, and vigorous physiological metabolism, which makes it very easy to lose water and wilt, yellowing and rotting, resulting in serious loss of nutrients and commercial value, and shortening of the shelf-life after harvesting. Post-harvest losses of vegetables are the highest of all kinds of food commodities globally, ranging from 28 to 55% of total production (Karoney et al., 2024). Therefore, research efforts need to be conducted to reduce post-harvest losses, preserving their nutritional quality, and extend the shelf life of vegetables. Temperature is one of the most crucial factors in regulating the post-harvest physiological and biochemical processes of vegetables. Most vegetables are harvested at relatively high temperatures, retaining a significant amount of field heat. High temperature increases their respiratory intensity and the growth rate of microorganisms (Han et al., 2017), making them highly susceptible to quality deterioration during shelf sales. As a result, the storage period and shelf life of the vegetables are significantly shortened. Therefore, they belong to a category of perishable products that are particularly challenging to maintain freshness in the fresh agricultural produce market. Therefore, it is essential to eliminate the field heat from agricultural produce immediately after harvest.

The purpose of pre-cooling is to eliminate field heat from freshly harvested vegetables, cool them to the appropriate temperature after harvesting and before transporting them to cold storage warehouses or markets, slow down the physiological and biochemical activities of the vegetables, and prepare for subsequent storage or shelf life (Zhang et al., 2023). In addition, pre-cooling can preserve fruit and vegetables from physiological disorders, postpone aging or maturation, decrease post-harvest spoilage, and conserve vegetable quality. Pre-cooling is the initial and most crucial step in post-harvest vegetable temperature management. It is the primary measure to slow down biological processes and preserve vegetable quality. Common pre-cooling techniques include natural convection pre-cooling, forced-air pre-cooling, vacuum pre-cooling, cold water pre-cooling, and ice pre-cooling. Although these pre-cooling methods all involve rapidly transferring heat from agricultural products to cooling media such as air and water, they differ technically. The most appropriate technique is dictated by specific parameters including the type of vegetables, container characteristics, cooling rate, and further storage and transportation conditions (Duan

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https://doi.org/10.1016/j.fochx.2024.101599
Received 12 March 2024; Received in revised form 24 June 2024; Accepted 25 June 2024
Available online 27 June 2024

et al., 2020). Many studies have focused on addressing the pre-cooling issues in overall post-harvest temperature management of vegetables, with relatively few studies on the optimal pre-cooling methods and quality changes during storage of bok choi. Therefore, this study mainly targets bok choi, using natural convection pre-cooling methods (NCPC), strong wind pre-cooling (SWPC), vacuum pre-cooling (VPC), cold water pre-cooling (CWPC), electrolyzed water pre-cooling (EWPC), and fluid ice pre-cooling (FIPC), extending the post-harvest storage period, and determining the most suitable pre-cooling preservation method, and providing a new solution for the most suitable preservation method for bok choi.

2. Materials and methods

2.1. Raw material treatment and pre-cooling method

Harvested bok choi from Lingang New City, Shanghai was quickly transported to the laboratory. Bok choi with consistent size, bright color, and no yellow leaves or diseases were selected. The selected bok choi was neatly arranged in plastic baskets to prepare for the following process. A thermocouple as a temperature sensor was inserted into the center of the root stem of bok choi to monitor its core temperature during the pre-cooling process. The samples from each group began pre-cooling simultaneously. After pre-cooling was completed, they were quickly dried and immediately placed into a refrigerator set at 4 °C with a relative humidity of 80%. Every three days, measurements were taken on indicators such as weight loss rate, conductivity, hardness, color, total viable count (TVC), respiration rate, chlorophyll, titratable acid (TA), soluble sugar, soluble solids, Vitamin C (VC), total phenols (TP), malondialdehyde (MDA) and antioxidant enzyme activity of the bok choi samples. Different pre-cooling methods were analyzed as follows (Zhang et al., 2023), and all vegetables were cooled from the initial temperature of 25 °C to 4 °C.

2.1.1 RT: Storing freshly harvested green vegetables without any precooling treatment at room temperature.

2.1.2 NCPC: The vegetables were placed in a refrigerator with natural cold air to eliminate heat from the product.

2.1.3 SWPC: Precooling with a wind speed of 7.4 m/s, air temperature of 0 °C, initial temperature of 25 °C, and final temperature of 4 °C by a forced-air machine (Pr.c-15, Shaanxi, China).

2.1.4 VPC: The vegetables were placed inside a vacuum chamber of the vacuum machine (VAC-0.2 type, Shanghai, China). The corresponding cooling final temperature is 4 °C, and the final pressure of 0.8 kPa. Spraying water with the weight of 4% of the vegetables in a vacuum chamber was added, and the change in pressure inside the vacuum chamber was also recorded.

2.1.5 CWPC: The vegetables were cooled down in a 200-l rectangular plastic water tank filled with cold water until the temperature dropped to 4 °C. The temperature of the cold water was maintained at 0 °C throughout the cooling process.

2.1.6 EWPC: Slightly acidic electrolyzed water was prepared using an electrolyzing machine (TTK-WEA100, Shanghai, China), and the water temperature was maintained at 0 °C.

2.1.7 FIPC: Preparing fluid ice using a fluidized ice maker (RF-1000 W-SP, Jiangsu, China). The fluid ice was injected into a foam box containing vegetables to perform precooling.

2.2 Weight loss, moisture content, hardness, color.

The weighing method was used and the surface of the bok choi was dried before weighing. The weight loss was calculated by the formula and the results were expressed in %. The formula is as follows:

$$\text{Weight loss }(\%) = \frac{\text{Initial mass of bok choi} - \text{the storage mass of the bok choi}}{\text{Initial mass of bok choi}} \times 100$$

After removing the stems from vegetables at room temperature, 1 g of leaf samples is taken. Subsequently, the leaf samples are placed on a clean platform of a moisture analyzer (HX204, METTLER TOLE-DO®) and dried to a constant weight. The moisture loss of the sample is calculated as the difference between its weight before drying (x) and its weight after drying (z). The moisture content is calculated as the ratio of the moisture loss to the original weight of the sample(Shi et al., 2021). The calculation formula is as follows:

$$\text{Moisture content }(\%) = \frac{(x - z)}{x} \times 100\%$$

The hardness was determined using a texture analyzer (TA-XT Plus C, Stable Micro Systems Co. Ltd., Surrey, UK). Hardness was measured using a texture analyzer with a probe diameter of 5 mm. The probe moved at a speed of 1.0 mm/s. The experimental penetration depth was 10 mm. Take two leaf slices from each bok choi, avoid the main veins, and symmetrically select two points on the upper part of the leaf. Use a colorimeter (CR-400, Konica Minolta, Tokyo, Japan) to measure the brightness (L* value), the redness-greenness (a* value), and the yellowness-blueness (b* value) of the bok choi leaves. ΔL represents the change in brightness of the sample compared to day 0, Δa represents the change in red-green chromaticity of the sample compared to day 0, and Δb represents the change in yellow-blue chromaticity of the sample compared to day 0. L0 is the brightness value of the sample on day 0, a0 is the red-green chromaticity value of the sample on day 0, and b0 is the yellow-blue chromaticity value of the sample on day 0. The color difference is calculated according to the following formulaΔE:

$$(\Delta E =)\sqrt{\left(\Delta L^2 + \Delta a^2 + \Delta b^2\right)^2} = \sqrt{\left((L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2\right)}$$

*2.2. **Relative** electrolyte leakage, total viable count (TVC), respiration rate*

Use a puncher to extract 2 g of round vegetable slices (5 mm diameter). The samples were positioned in test tubes containing 30 mL of distilled water at 25 °C. The initial conductivity (E1) was determined using a digital conductivity meter (DDS-307 A, INESA®). After 2 h of standing, the ultimate conductivity (E2) was determined again after boiling for 20 min and cooling to 25 °C. The relative electrolyte leakage was calculated by the formula:

$$\text{Relative electrolyte leakage} = \frac{\text{initial conductivity (E1)}}{\text{total conductivity (E2)}} \times 100$$

5 g of bok choi sample was combined with 45 mL of 0.85% sterile saline and shaken vigorously, followed by graded dilutions of 0.85% sterile saline to 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}, respectively, and the sample dilutions were dropped onto nutrient agar plates for colony counting under aseptic conditions and incubated at 37 °C for 72 h.

Respiration rate was determined by static method, the sample was placed in a desiccator, the bottom of the desiccator was put into a 0.4

mol/L NaOH solution 20 mL of quantitative lye, the CO_2 released by the respiration of vegetables sunk and was absorbed by the lye, after a certain period of time after static take out the lye, add saturated $BaCl_2$ 5 mL, 2 drops of phenolphthalein, titration with 0.1 moI/L oxalic acid, the respiratory strength of samples was found.

2.3. Chlorophyll, TA, soluble sugar, soluble solids

The determination of total chlorophyll, TA, and soluble sugar content was conducted using the Solabao test kits for chlorophyll, TA, and soluble sugar content. The reagent kit determines TA content through titration. Soluble sugar content is determined using the anthrone colorimetric method. The determination of soluble solids was carried out according to the method described by Li et al. (Li et al., 2024). Grind 5 g of the sample thoroughly, take 1 drop of the supernatant, and measure the mass fraction of soluble solids using a digital refractometer (PR32a, ATAGO, Japan).

2.4. Vitamin C (VC), total phenols (TP), malondialdehyde (MDA)

VC, TP, and MDA were determined using the Beijing Solarbio VC, TP, and MDA test kits. The VC content is determined by reducing Fe^{3+} to Fe^{2+}, followed by a color reaction with ferroin and measuring the absorbance at 536 nm. The total phosphorus (TP) content is determined by reducing tungstomolybdic acid and measuring the absorbance at 760 nm. Malondialdehyde (MDA) is estimated by reacting with thiobarbituric acid and measuring the absorbance at 532 nm.

2.5. Antioxidant enzyme activity (superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT))

SOD, APX, and CAT activities are determined using the Beijing Solarbio SOD, APX, and CAT enzyme assay kits. The SOD assay kit uses the WST-1 (Water-Soluble Tetrazolium salt-1) method. Superoxide anions (O_2-) are generated by a reaction system involving xanthine and xanthine oxidase. O_2- reacts with WST-1 to produce a water-soluble yellow formazan dye, which has an absorption peak at 450 nm. SOD scavenges O_2-, thereby inhibiting formazan formation. SOD activity is measured by assessing the absorbance of the reaction mixture. APX catalyzes the oxidation of ascorbate (AsA) by H_2O_2. APX activity is determined by measuring the rate of AsA oxidation. H_2O_2 exhibits a characteristic absorption peak at 240 nm. CAT decomposes H_2O_2, causing a decrease in absorbance at 240 nm over time in the reaction solution. CAT activity can be calculated based on the rate of absorbance change.

2.6. Polyphenol oxidase (PPO), peroxidase (POD)

PPO and POD enzyme activities are determined using the Beijing Solarbio PPO and POD enzyme activity assay kits. PPO catalyzes the production of ortho-quinone from catechol. PPO enzyme activity is measured by the characteristic light absorption of ortho-quinone at 410 nm. POD catalyzes the oxidation of specific substrates by H2O2, exhibiting characteristic light absorption at 470 nm. Determine POD enzyme activity by measuring its absorbance values.

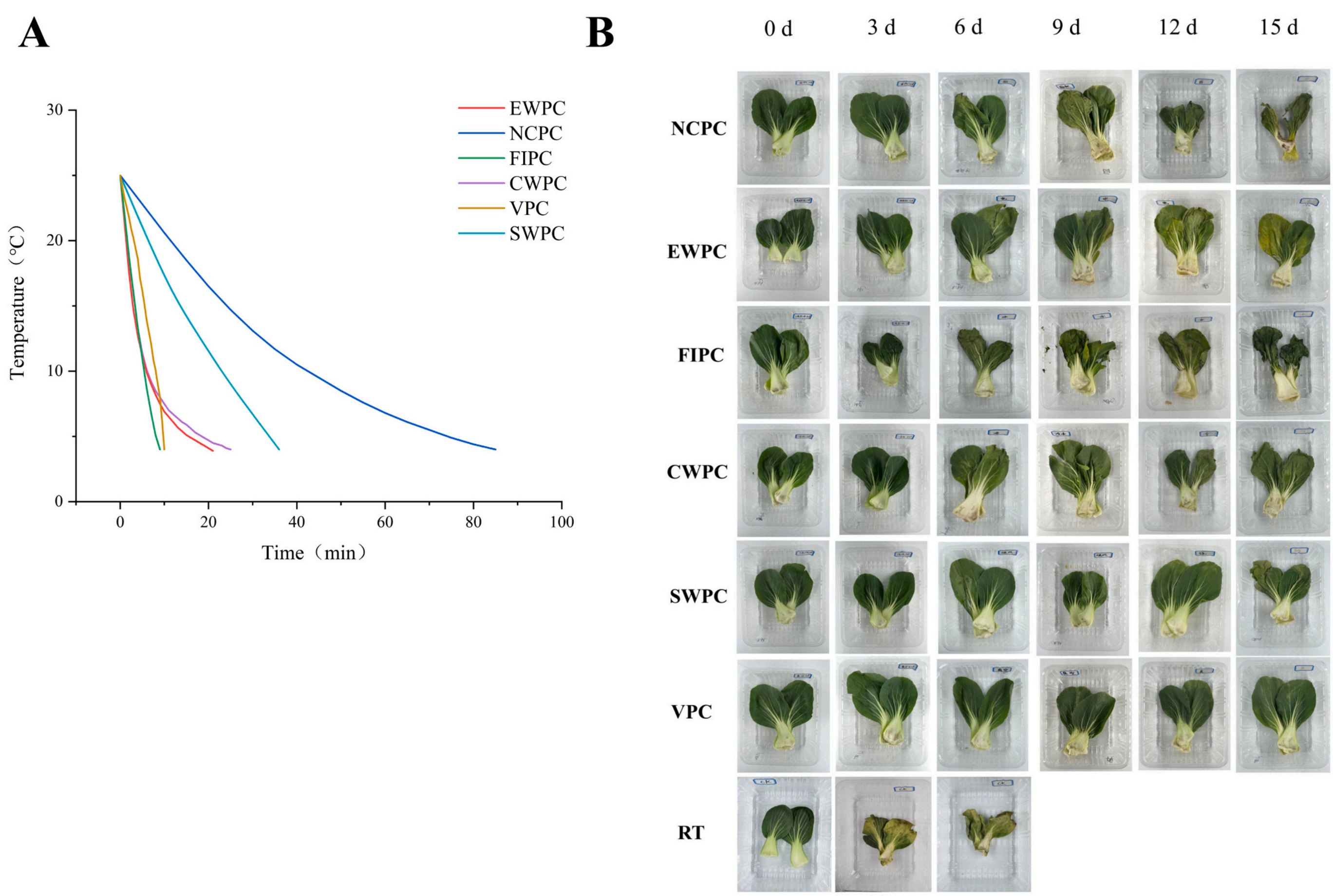

Fig. 1. Changes in (A) pre-cooling curve and (B) appearance of bok choi with different pre-cooling methods during cold storage. The abbreviations RT, EWPC, NCPC, FIPC, CWPC, VPC, and SWPC in the figure correspond to the room temperature group, slightly acidic electrolyzed water pre-cooling, natural convection pre-cooling, fluid ice pre-cooling, cold water pre-cooling, vacuum pre-cooling, and strong wind pre-cooling, respectively.

2.7. Statistical analysis

All data were repeated three times and expressed as mean standard deviation. Independent samples *t*-test, one-way analysis of variance (ANOVA), and correlation analyses were performed using SPSS 20.0, and $P < 0.05$ was considered significant. Origin 2018 was used for plotting.

3. Results and discussion

3.1. Temperature changes of grapes during precooling and photos during storage

After harvesting, pre-cooling is beneficial for delaying the deterioration of bok choi. Different pre-cooling methods have varying cooling speeds, with VPC being faster and causing less damage to the bok choi (McDonald & Sun, 2000). As shown in Fig. 1(A), all samples were cooled from 25 °C to 4 °C. Despite FIPC having the shortest cooling time, it still led to bok choi deterioration due to cold injury (Alabi et al., 2020; Wu et al., 2024). In addition to FIPC, VPC samples cool at a faster rate than other samples, facilitating the rapid release of latent heat in bok choi. This helps to suppress respiration and transpiration (Xanthopoulos et al., 2017), thereby maintaining better storage quality. On the contrary, NCPC treatment has the longest pre-cooling time and cannot rapidly release latent heat. This issue also exists in the SWPC treatment group, possibly owing to insufficiently low wind temperature. However, if the wind temperature is further reduced, the quality of the bok choi will deteriorate further. Alibas et al. (Alibas & Okursoy, 2016) studied the impact of various pre-cooling techniques on the quality of artichokes. Their research suggests that VPC has the shortest pre-cooling time and provides the best preservation effect on artichoke quality, followed by CWPC. That aligns closely with our experimental results.

Figs.1(B) illustrate the appearance photographs of bok choi during refrigeration. The photos indicate that the quality of bok choi deteriorated with prolonged storage time. During refrigeration, both the NCPC group and the FIPC treatment group experienced poor appearance due to yellowing and wilting of the bok choi surface. The FIPC treatment group showed wilting from the 3rd day, and the degree of wilting remained severe thereafter. This may be due to the precooling process of FIPC treatment damaging the tissue structure of bok choi, leading to chilling injury (Alabi et al., 2020). The EWPC and CWPC treatment groups exhibited yellowing and mild wilting in the later stages of refrigeration. During the refrigeration process, yellowing and wilting are two important factors leading to the deterioration of the post-harvest

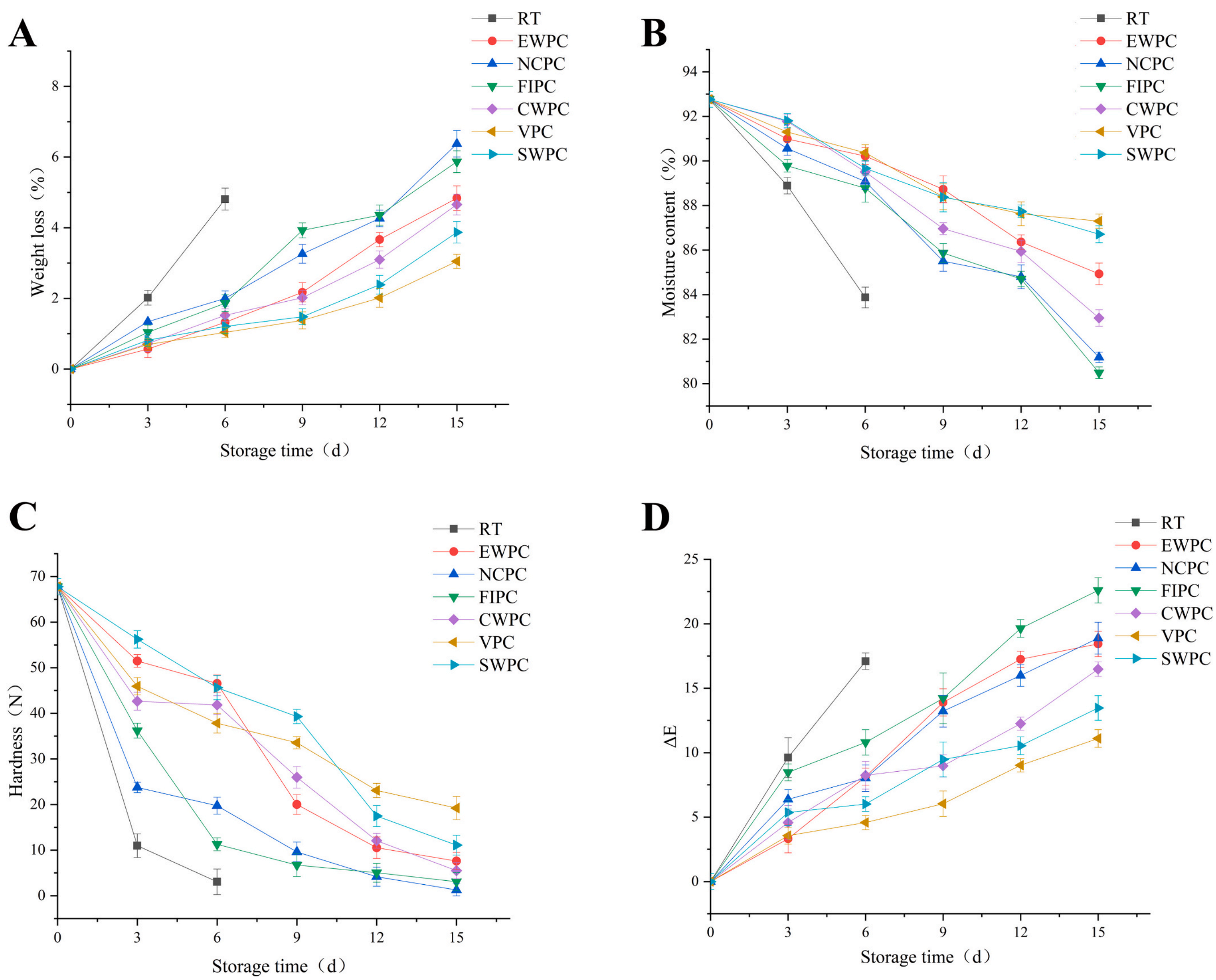

Fig. 2. Changes in (A) weight loss, (B) moisture content, (C) hardness, and (D) color of bok choi with different pre-cooling methods during cold storage. The abbreviations RT, EWPC, NCPC, FIPC, CWPC, VPC, and SWPC in the figure correspond to the room temperature group, slightly acidic electrolyzed water pre-cooling, natural convection pre-cooling, fluid ice pre-cooling, cold water pre-cooling, vacuum pre-cooling, and strong wind pre-cooling, respectively.

storage quality of vegetables. Bok choi in the VPC and SWPC groups exhibit less yellowing and wilting, indicating that VPC and SWPC treatments alleviate these phenomena. The bok choi treated with VPC maintained a good appearance at the end of refrigeration (15 days) and outperformed the other experimental groups. This may be owing to the fast cooling speed of VPC, which quickly removes the field heat from the bok choi, reducing their respiration and transpiration rates.

3.2. Weight loss, moisture content, hardness, color

Weight loss is a crucial factor in assessing the freshness of vegetables, especially bok choi, which has a high water content. After harvesting, bok choi continues to undergo strong transpiration and respiration during storage, making it susceptible to dehydration and wilting, which can lead to a loss in commercial value. (Gomes et al., 2023). During postharvest storage of bok choi, weight loss of bok choi was affected by different pre-cooling treatments and storage time. Fig. 2(A) indicated that as the storage time increased, the weight loss rate of bok choi showed a gradual upward trend, but different precooling methods had varying degrees of inhibitory effects on it. As early as the third day, the weight loss rate of non-precooled bok choi was higher than that of precooled bok choi. This may be due to the fact that the high metabolic state of the non-precooled bok choi mainly involves respiratory processes during storage, leading to a higher early-stage weight loss rate during storage. In general, transpiration and respiration are the two main processes involved in water loss from fresh produce. This occurs as a result of the difference in vapor pressure between the fruit and its environment (Xanthopoulos et al., 2017). The higher weight loss of bok choi treated with NCPC may be due to the longer precooling time, the vapor pressure difference between the vegetable and the surrounding environment, and the high transpiration and respiration caused by bok choi's field heat. The FIPC treatment group experienced higher weight loss due to cold injury damaging the tissue structure of bok choi, while simultaneously stimulating the metabolic state of bok choi (Alabi et al., 2020; Wu et al., 2024). Both the EWPC and CWPC treatment groups experienced weight loss exceeding 4% at the end of storage (15 days). Although water cooling can rehydrate and restore agricultural products, it may also result in excessive water absorption, leading to cell rupture and subsequent weight loss during the storage process. Compared to the others, bok choi treated with VPC experienced the lowest weight loss ($p < 0.05$)and had the longest storage time. Ding et al. (Ding et al., 2016) also found that vacuum pre-cooling combined with water replenishment did not have any negative impact on the quality of the vegetables compared to several other cooling methods, due to the fact that spraying water on the vegetables greatly reduced the weight loss during vacuum cooling. This indicates that combining VPC treatment with appropriate watering can effectively reduce the transpiration and respiration of bok choi, thereby decreasing the weight loss rate.

Water is the solvent for all physiological and biochemical reactions inside vegetables, playing a unique role in metabolic processes. Therefore, the moisture content is a crucial indicator of the freshness of vegetables. Due to its large surface area, bok choi evaporates moisture quickly after harvest, making it highly susceptible to wilting and causing a decrease in its commercial value. Fig. 2(B) shows a decreasing trend in water content during storage, which was attributed to the loss of bok choi moisture due to the respiration of bok choi and the relative humidity of the environment (Zuo et al., 2021). During the early stage of storage, the moisture content of all experimental groups was higher than that of the RT group, indicating that post-cooling followed by low-temperature storage can effectively reduce moisture loss during the storage process. After 9 days of storage, the moisture content of the groups treated by VPC and SWPC remained at a relatively high level ($P < 0.05$), indicating that VPC and SWPC treatments can effectively inhibit the transpiration and respiration of bok choi, thereby effectively delaying the moisture loss of bok choi during storage.

Hardness is one of the most important indicators for evaluating the ripeness and freshness of vegetables. Hardness reflects the textural characteristics of vegetables and is related to cell wall composition and cell wall-degrading enzyme activity. Fig. 2(C) shows the hardness changes of bok choi during storage. Using different pre-cooling methods to treat bok choi resulted in varying changes in hardness during cold storage. The hardness of each treatment group exhibited a decreasing trend, with the bok choi texture transitioning from crisp and firm to loose and soft, possibly due to cellular dehydration in bok choi. This dehydration could lead to cell shrinkage and separation, ultimately causing the bok choi to appear collapsed and wilted, resulting in a continuous decrease in its hardness. In the experimental group, bok choi treated with FIPC exhibited the fastest decline in hardness, possibly due to low temperatures damaging the tissue structure of bok choi (Farneti et al., 2015), leading to a decrease in hardness. The group treated by VPC maintained a higher level of hardness during the storage period, with the highest hardness observed at the end of the storage period (15d) ($P < 0.05$). This indicates that VPC has the greatest impact on preserving the hardness of bok choi during the storage period.

The color of vegetables is one of the key external features used to evaluate their post-harvest life, and it is an essential factor that influences their sensory quality and shelf life, as well as being a primary factor in consumer purchasing decisions. Human perception of color is highly complex and observations of brightness, intensity, luminance, and vividness can all alter their perception of color. Therefore, color characteristics are crucial as they can influence the consumers' perception of fruit quality and preference. The color can be represented by the color difference (ΔE), with a larger ΔE value indicating a greater color change. Fig. 2(D) depicts the changes in color difference (ΔE) of bok choi in each group during the storage period. In the first 3 days, except for the RT group, the FIPC-treated group showed the greatest increase. This may be due to the damaging effect of fluid ice precooling on the cell membrane permeability of bok choi, resulting in the loss of pigment substances and the occurrence of enzymatic browning in bok choi (Balwinder et al., 2018). Additionally, in the later stages, the enhanced respiratory activity in all groups accelerated the rapid decomposition of pigments. The VPC-treated group exhibited overall lower color differences compared to the other groups, with the lowest color difference observed at the end of the storage period (15d) ($p < 0.05$). This may be attributed to the VPC treatment attenuating the respiratory activity of bok choi, thereby reducing the rate of pigment decomposition. These findings suggest that the VPC treatment has a certain mitigating effect on the color difference changes during the storage period of bok choi. Another study has found similar results, with the color of cauliflower being optimal with VPC treatment (Alibas et al., 2015).

3.3. Relative electrolyte leakage, TVC, respiration rate

In the physiological and metabolic processes of vegetable tissues, the level of cell membrane permeability reflects the integrity and stability of the cell membrane, serving as an important indicator of freshness. The preservation of harvested vegetables is heavily reliant on the integrity of their cell membranes, which plays a crucial role in controlling the browning process. When the membrane integrity is impaired, the direct interaction between substrates, phenols, and browning-related enzymes leads to the accumulation of quinones and browning. Therefore, cell membrane permeability is the most commonly applied indicator for membrane condition. In Fig. 3(A), the relative electrolyte leakage is used to evaluate the integrity of the cell membrane. With the extension of storage time, the relative electrolyte leakage of bok choi showed an upward trend. This is because, during the storage process, the membrane permeability of bok choi increases, leading to a significant leakage of electrolytes from the cells. In the early stage of storage, except for the RT and FIPC-treated groups, the other groups showed a slight increase in relative electrolyte leakage of bok choi. The significant increase of

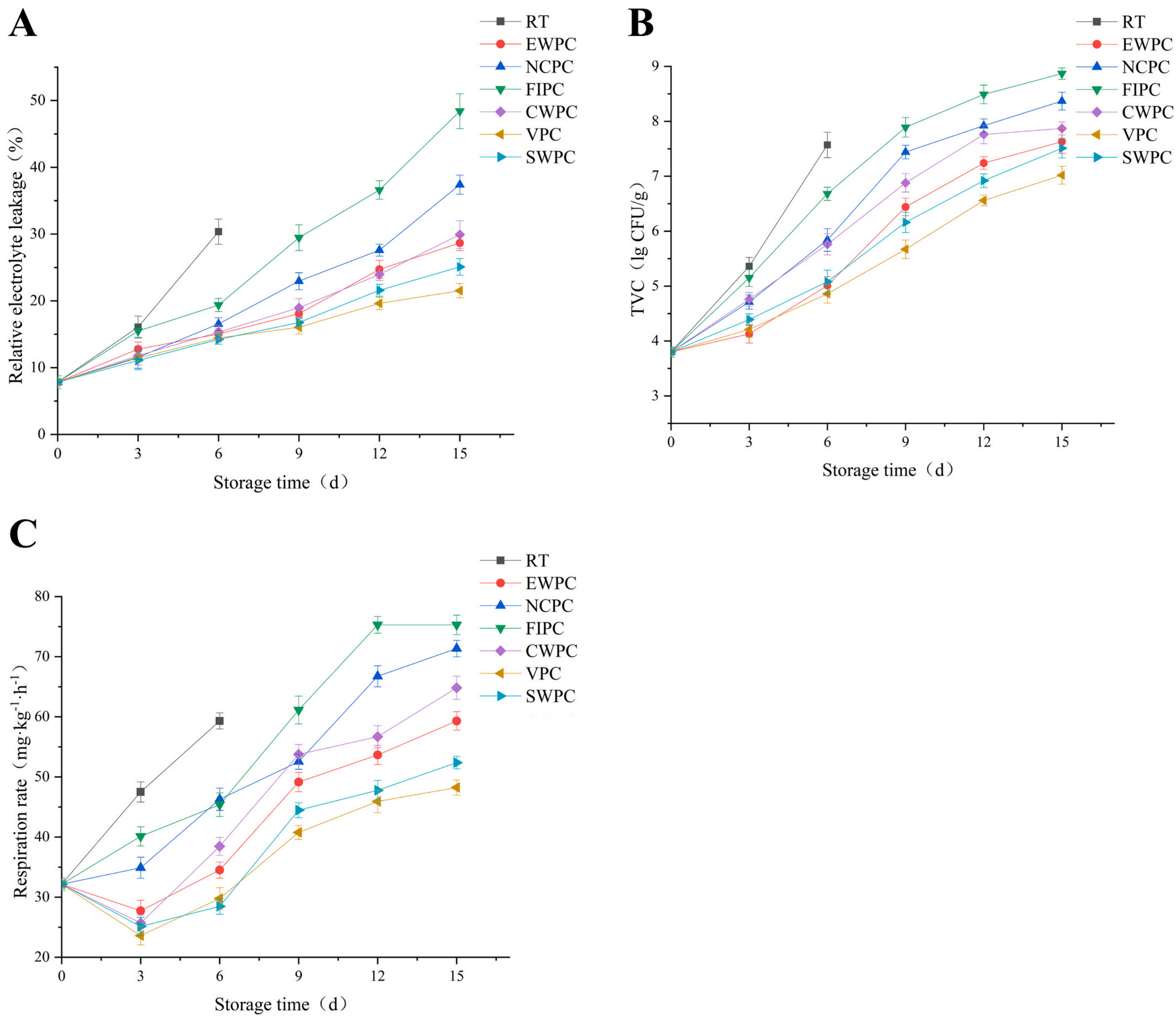

Fig. 3. Changes in (A) relative electrolyte leakage, (B) TVC, and (C) respiration rate of bok choi with different pre-cooling methods during cold storage. The abbreviations RT, EWPC, NCPC, FIPC, CWPC, VPC, and SWPC in the figure correspond to the room temperature group, slightly acidic electrolyzed water pre-cooling, natural convection pre-cooling, fluid ice pre-cooling, cold water pre-cooling, vacuum pre-cooling, and strong wind pre-cooling, respectively.

relative electrolyte leakage in the FIPC-treated precooling group may be attributed to the cold injury caused by low temperature and FIPC treatment, resulting in tissue damage to bok choi cells (Alabi et al., 2020; Wu et al., 2024). This damage leads to the disruption of cell membrane integrity and the significant leakage of electrolytes, accelerating the aging process of the fruit. In the later stage of storage (15 days), the relative electrolyte leakage of bok choi by VPC treatment was the lowest ($p < 0.05$), indicating that VPC treatment can effectively protect cell membrane permeability and thus delay the aging process of bok choi.

TVC is the most important indicator for assessing food safety in fresh-cut fruits and vegetables. Excessive microbial counts can lead to spoilage and deterioration, which is not only the main reason for judging the unqualified quality of fruits and vegetables but also a crucial factor affecting their shelf life. According to Fig. 3(B), during the storage period, the TVC of all groups increased continuously. Among them, the RT group had the fastest rate of increase, which may be attributed to the effective reduction of enzyme system activity in microorganisms through precooling and refrigeration treatment, thereby slowing down the metabolism and reproduction rate of microorganisms. In the early stage of storage, apart from the RT group, the FIPC-treated group showed the fastest rate of increase. This may be due to the cold injury caused by FIPC treatment, which damages the cells on the surface of bok choi (Alabi et al., 2020; Wu et al., 2024), increases cell membrane permeability, and leads to the leakage of cell contents. This provides favorable conditions for microbial growth and reproduction； The significant increase in the CWPC-treated group may be because, after CWPC treatment, although the surface moisture was thoroughly wiped off, the layered wrapping of the root stems of bok choi may have led to residual moisture inside the heart of the vegetable. This residual moisture can serve as an important factor for nourishing microorganisms, leading to microbial proliferation and eventually causing decay and spoilage of bok choi (Brosnan & Sun, 2001; Zhu et al., 2019). The minimal increase in the early storage period for the EWPC-treated pre-cooling group and the VPC-treated group, reaching 5.01 lg CFU/g and 4.86 lg CFU/g on the 6th day of storage, could be due to the potential sterilizing effect of EWPC treatment and pre-cooling, as well as VPC treatment. He et al. (He et al., 2013) confirmed through scanning electron microscopy that the vacuum cooling process caused significant damage to the membrane structure of *Escherichia coli*, resulting in shrinkage and roughness. Therefore, they believe that the inactivation of microorganisms by VPC is mainly caused by DNA damage, which disrupts the cell's reproductive capacity and other functions. In addition, the VPC treatment greatly reduces the possibility of microbial contamination during the pre-cooling process of bok choi because it does not come into contact with any medium other than air. Research has found that compared to traditional pre-cooling methods, VPC treatment can significantly reduce the cooling time for mushrooms and lower the rate of microbial growth (Zhang et al., 2018). Furthermore, during the later stages of storage, the TVC of the VPC-treated group consistently remained at a lower level. After 15 days of storage, the TVC content was the lowest ($p < 0.05$), indicating that VPC treatment is the most effective pre-cooling method for inhibiting bacterial growth in bok choi.

The magnitude of the respiration rate of vegetables after harvest is a crucial factor that affects post-harvest preservation time. Through respiration, vegetables break down complex organic substances into simpler compounds to generate energy (Wang et al., 2022). Therefore, the faster the respiration rate, the faster the loss of vegetable quality, and the shorter the preservation period. From Fig. 3(C), it can be observed that the respiration rate of bok choi generally shows an upward trend during the storage period, which is consistent with the findings of Cai et al.'s (Cai et al., 2024) study. During the first three days of storage, except for the RT group, the FIPC-treated group showed the highest upward trend in respiration rate, which may be due to the low-temperature damage that disrupted the tissue cells of bok choi and stimulated its respiration, resulting in increased respiratory intensity. The slight downward trend observed in the other groups during the first three days may be attributed to the inhibitory effects of low temperature and pre-cooling on the respiration of bok choi (Wang et al., 2021). After the 6th day, the respiration rate of the VPC-treated group and SWPC-treated group was significantly lower than those of the other groups ($p < 0.05$), indicating that vacuum and SWPC treatments can rapidly eliminate field heat in bok choi and reduce its respiration rate. Garrido

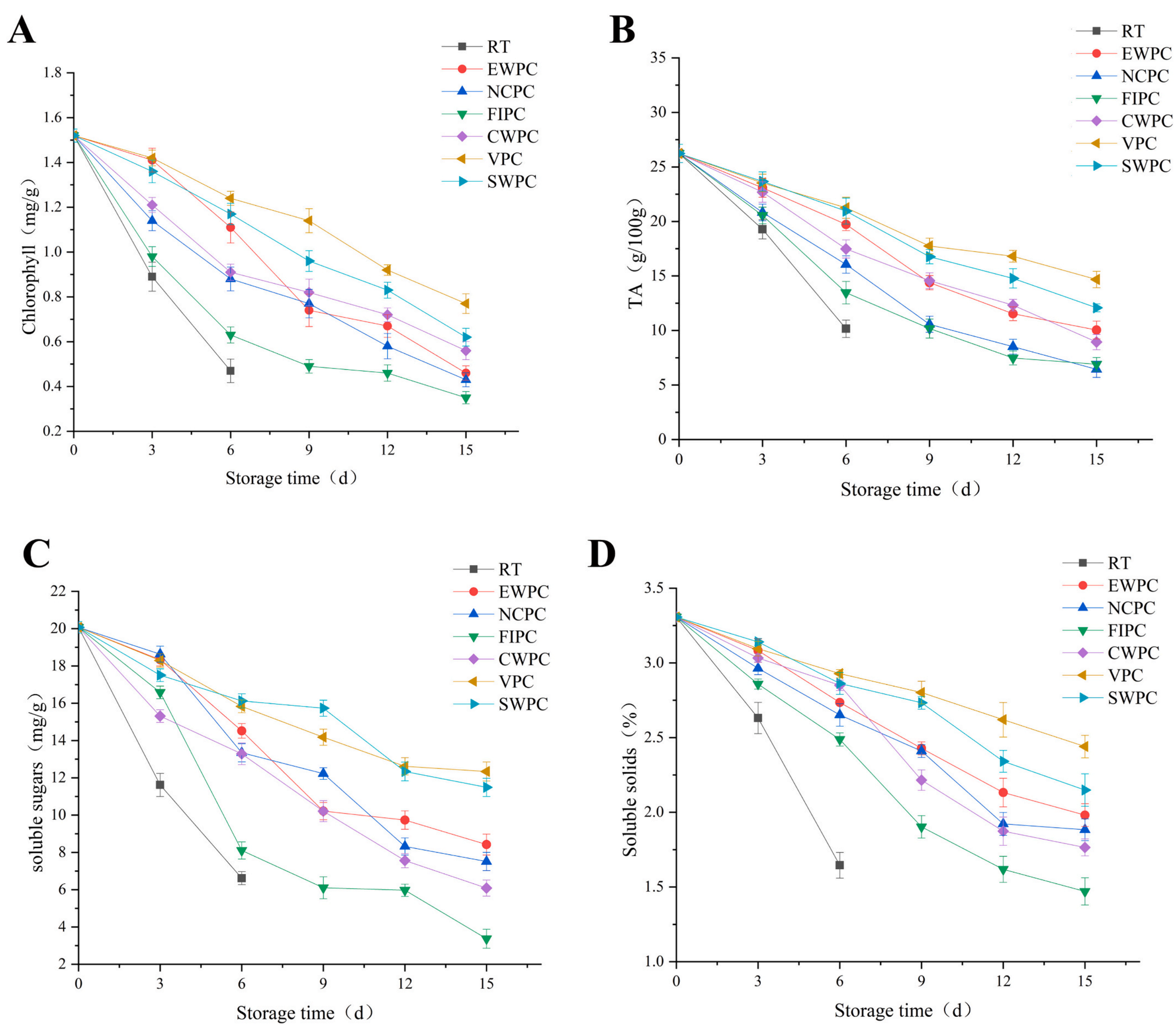

Fig. 4. Changes in (A) Chlorophyll, (B) TA, (C) soluble sugars, and (D) soluble solids of bok choi with different pre-cooling methods during cold storage. The abbreviations RT, EWPC, NCPC, FIPC, CWPC, VPC, and SWPC in the figure correspond to the room temperature group, slightly acidic electrolyzed water pre-cooling, natural convection pre-cooling, fluid ice pre-cooling, cold water pre-cooling, vacuum pre-cooling, and strong wind pre-cooling, respectively.

et al. (Garrido et al., 2015) compared the effects of different cooling methods on the preservation efficiency of spinach and also found that VPC treatment could effectively inhibit respiration intensity.

3.4. Chlorophyll, titratable acids(TA), soluble sugars, soluble solids

Chlorophyll levels are a crucial indicator of plant physiological activity, which not only affects the photosynthesis of plants but also influences the quality and sales of vegetables. The green color presented in the leaves of bok choi is determined by the chlorophyll and its derivatives in the leaves. When bok choi is affected by adverse environmental factors, chlorophyll is catalyzed by enzymes or undergoes a photodynamic oxidation reaction under light, resulting in the formation of colorless substances (Sun & Li, 2017). Fig. 4(A) shows the changes in the total chlorophyll content of bok choi in each group during the storage period. From the figure, it was evident that the chlorophyll content in each group of bok choi continuously decreased during the storage process due to degradation. The VPC treatment group maintained a total chlorophyll content of 0.77 mg/g at day 15 of storage, which was significantly higher than the other groups ($p < 0.05$). This only represented a 49.34% decrease compared to the fresh samples. Furthermore, this group consistently maintained a relatively higher chlorophyll content throughout the storage period. This may be attributed to the beneficial effects of VPC treatment in maintaining the activity of relevant endogenous antioxidant enzymes, thereby slowing down the degradation of chlorophyll in bok choi and reducing the accumulation of free radicals (Huang & He, 2023). It can be seen that VPC treatment can effectively delay the degradation of total chlorophyll in bok choi. This was similar to the results of Zhang et al.'s (Zhang et al., 2021) study on VPC-treated mung beans.

TA content is an important factor affecting the flavor of vegetables. Vegetables contain various organic acids such as malic acid, citric acid, and tartaric acid. TA content content has an impact on the flavor, storage stability, and processability of vegetables. Additionally, the TA content of vegetables changes continuously with their maturity; as acidity decreases, the quality of vegetables also decreases (Anthon & Barrett, 2012). Fig. 4(B) shows the variation in TA content of bok choi during chilling by different pre-cooling treatments. According to Fig. 4(B), it can be observed that there is a gradual decrease in the TA content of bok choi with prolonged storage time. This is due to the consumption of organic acids as respiratory substrates and the decarboxylation reaction of pyruvic acid during the storage period, leading to a decrease in acid content (Chen et al., 2023). After 6 days of storage, the VPC and SWPC treatment groups had significantly higher TA content compared to the other groups ($p < 0.05$). Xinyu et al. (Xinyu et al., 2023) also found that VPC treatment maintained higher TA content in cabbage, which may be due to the fact that VPC treatment reduces organic acid consumption by inhibiting the respiration rate and delaying the ripening process in bok choi. Bok choi treated with VPC showed the least variation in TA content. After 15 days of storage, it only decreased by 44.05% compared to the initial value. This indicates that VPC treatment can effectively delay the decrease in TA content in bok choi.

Soluble sugars in fruits and vegetables are mainly glucose, fructose, and sucrose. They not only determine the flavor and texture of fruits and vegetables, but also closely related to physiological activities during the storage period. According to Fig. 4(C), it can be observed that the soluble sugar content of bok choi in all groups decreases during the storage period. Among them, bok choi treated with VPC and SWPC showed significantly higher soluble sugar content than other treatments ($P < 0.05$) from 9 to 15 days of storage. This indicates that VPC and SWPC treatments can effectively delay the decrease in soluble sugar content.

The soluble solids in bok choi serve as an indicator of sweetness, and their content level can be used as one of the indicators to evaluate its quality and freshness preservation effects (Shan et al., 2023). According to Fig. 4(D), it can be observed that the soluble solid content of bok choi in all groups gradually decreases with the prolonging of storage time. This may be due to the extensive microbial propagation and the consumption of sugars and other substances by the inherent metabolism of bok choi, leading to a reduction in the soluble solid content in bok choi. Among them, the VPC treatment group exhibited the slowest decline rate. This indicates that VPC treatment can effectively delay the decrease in soluble solid content of bok choi during storage. Juan et al. (Juan et al., 2020) also found that compared with other pre-cooling methods, VPC treatment can effectively delay the deterioration and nutritional loss of sensory quality indicators such as vitamin C ($P < 0.05$) and soluble solids ($P < 0.05$) during storage. They believe that this is mainly because VPC treatment effectively suppresses the respiration rate and nutrient metabolism of bok choi during storage, reducing the consumption of soluble solids.

3.5. VC, TP,MDA

VC is an unstable vitamin and an important nutritional component in vegetables. As an antioxidant, it has the effect of scavenging free radicals, preventing aging, and preventing scurvy in the human body. Moreover, VC has antioxidant properties and can eliminate the damage caused by reactive oxygen species, thus delaying the aging of vegetables. The level of its content can be used as a basis for evaluating the quality of vegetables. Therefore, the content of VC is an important indicator for judging the quality of fruits and vegetables. According to Fig. 5(A), it can be seen that during the storage process, the VC content of bok choi in all groups gradually decreases due to enzymatic oxidation (Juan et al., 2020). At the end of the storage period, the VPC treatment group showed the lowest rate of VC loss ($p < 0.05$), which may be attributed to the VPC treatment delaying the respiration of bok choi, suppressing enzyme activity, microbial growth, and subsequently slowing down the decrease in VC content. This is similar to the results observed in cabbage treated with VPC (Ya et al., 2016). It can be seen that VPC treatment provides the best protection for VC.

Vegetables contain abundant phenolic compounds, which are important secondary metabolites in the growth, development, and ripening processes. They also serve as crucial antioxidants. TP is also one of the substrates for enzymatic browning in vegetables, and their levels can reflect the degree of browning to a certain extent. From Fig. 5(B), it can be observed that the total phenolic content of bok choi generally decreases with increasing storage time. In the VPC treatment group, it took 12 days for the total phenolic content to decrease to 3.54 mg/g. By the 15th day of storage, the VPC treatment group had the highest total phenolic content at 3.25 mg/g, which was 85% of the initial value and significantly higher than the other groups ($p < 0.05$). This indicates that VPC treatment can inhibit the decrease in the total phenolic content of bok choi. This is similar to the results reported by Xinyu et al. (Xinyu et al., 2022).

MDA, as the final product of lipid peroxidation, is highly toxic to plant tissues. Its content is positively correlated with membrane lipid damage and the degree of tissue aging. It can be used to evaluate the extent of damage to the cell membrane system in fruits and vegetables (Shan et al., 2023). As shown in Fig. 5(C), the MDA content of bok choi generally shows an upward trend during storage, which is consistent with the gradual aging of bok choi during storage, the accumulation of MDA, increased membrane permeability, and deterioration of tissue aging quality. Among them, the amount of malondialdehyde (MDA) produced in the bok choi of the VPC treatment group was relatively minimal. At 15 days, it was only 25.7 nmol/g, significantly lower than those of the other treatment groups ($p < 0.05$). This indicates that VPC treatment can effectively inhibit the accumulation of MDA in post-harvest bok choi, protecting cell membranes and their functions from damage. According to reports, VPC treatment can delay the increase of MDA content in green peppers (Qing et al., 2019) and *Aralia elata* (Huan et al., 2016).

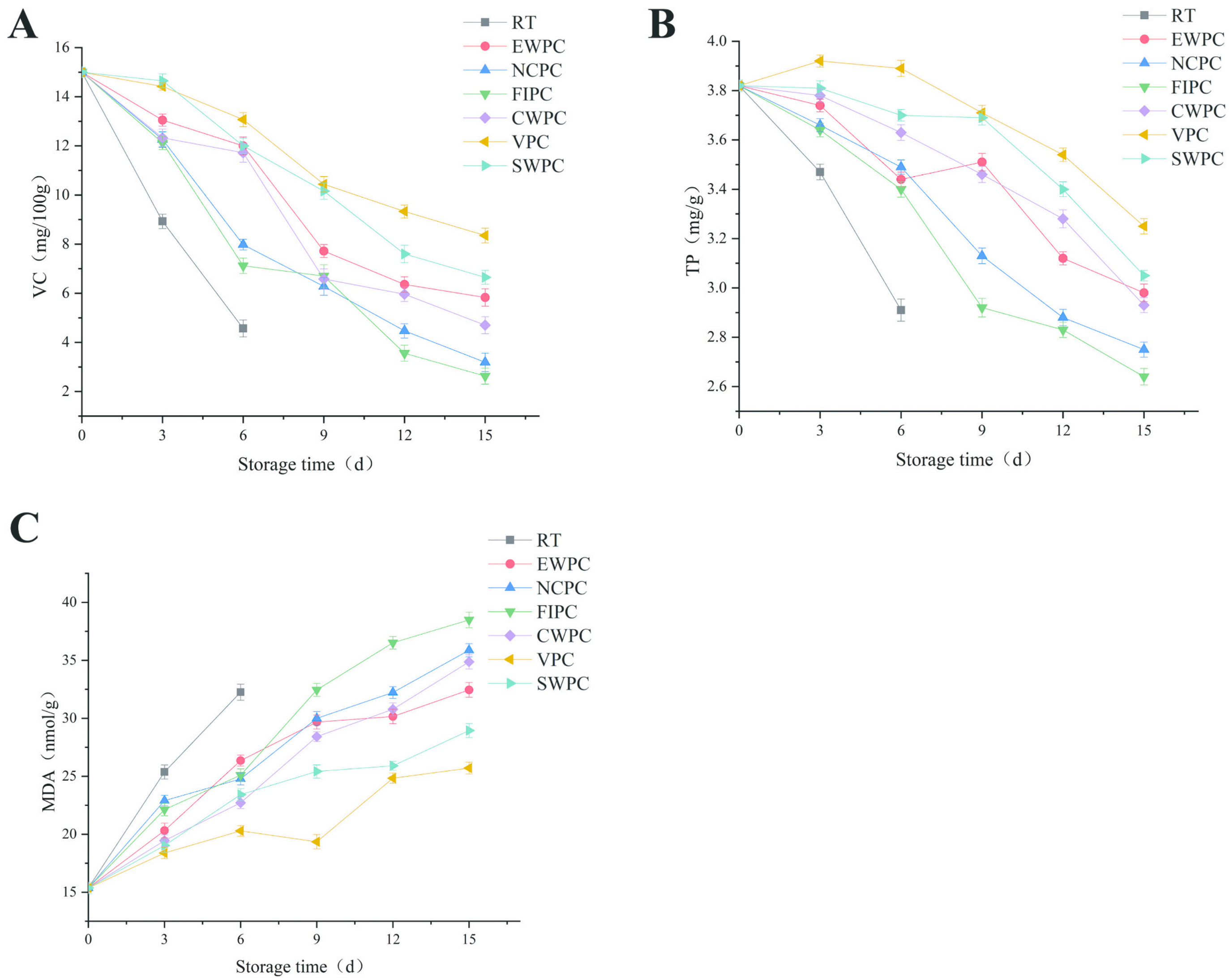

Fig. 5. Changes in (A) VC, (B) TP, and (C) MDA of bok choi with different pre-cooling methods during cold storage. The abbreviations RT, EWPC, NCPC, FIPC, CWPC, VPC, and SWPC in the figure correspond to the room temperature group, slightly acidic electrolyzed water pre-cooling, natural convection pre-cooling, fluid ice pre-cooling, cold water pre-cooling, vacuum pre-cooling, and strong wind pre-cooling, respectively.

3.6. Sod, APX, cat

Post-harvest oxidative stress is one of the key factors leading to the deterioration of fruit and vegetable quality. It is well known that the aging of plants is mainly due to the generation of reactive oxygen species (ROS), which cause oxidative damage to proteins and lipids in plant cells. During oxidative stress, the level of ROS exceeds the metabolic requirements, ultimately damaging biomolecules such as nucleic acids, proteins, and lipids, as well as various cellular structures and functions. Therefore, controlling the generation of ROS is an important strategy to delay post-harvest senescence and oxidative stress in vegetables. The elimination of reactive oxygen species can be categorized into two main types: antioxidant compounds and antioxidant enzymes. Antioxidant compounds include vitamin E, polyphenols, and carotenoids. Antioxidant enzymes mainly include SOD, APX, CAT, etc. (Zhang & Jiang, 2019). In the antioxidant enzyme system, SOD is the first line of defense in the antioxidant process for vegetables, responsible for converting superoxide anion radicals ($O_2^- \cdot$) into H_2O_2 and O_2. The subsequently generated H_2O_2 is then removed by CAT and APX. These enzymes are associated with the aging and stress response of plant tissues, and the effective activity of these antioxidant enzymes is crucial in preventing damage caused by ROS (Hodges et al., 2004). They can minimize free radical damage to ensure tissue survival under stress.

As shown in Fig. 6(A)(B)(C), during the storage process of bok choi, the activities of SOD, APX, and CAT all exhibit an initial increase followed by a decrease. The initial increase in activity may be a response to the elevated levels of reactive oxygen species during the early stages of storage, aiming to maintain balance. The subsequent decline in activity during the later stages of storage may indicate a disruption of the inherent regulatory mechanisms, leading to the impaired ability to effectively remove reactive oxygen species, which in turn negatively impacts the antioxidant enzymes, resulting in a decrease in enzyme activity. The lower activity of the FIPC-treated group during storage may be attributed to the disruption of the antioxidant system of bok choi due to cold stress. Additionally, it can lead to the accumulation of reactive oxygen species in vegetables, resulting in senescence and cell death (Junru & Min, 2021).

From Fig. 6(A), it can be observed that the SOD activity of the VPC-treated group and the SWPC-treated group peaked on the 9th day, and the SOD activity at the end of the storage period was significantly higher than those of the other groups ($p < 0.05$). From Fig. 6(B), it is evident that the APX activity of the VPC-treated group peaked on the 6th day,

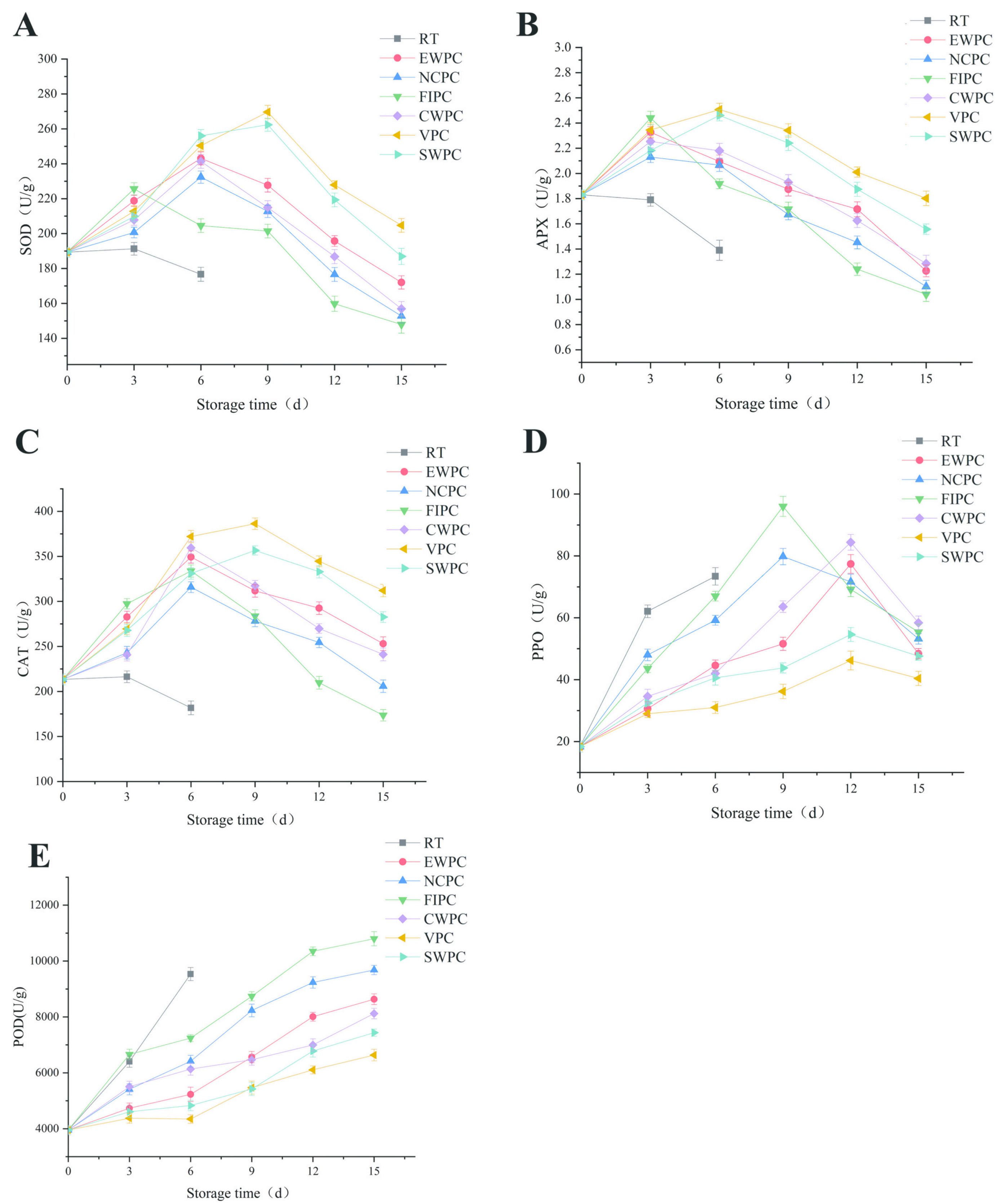

Fig. 6. Changes in (A) SOD, (B) APX, (C) CAT, (D) PPO, and (E) POD of bok choi with different pre-cooling methods during cold storage. The abbreviations RT, EWPC, NCPC, FIPC, CWPC, VPC, and SWPC in the figure correspond to the room temperature group, slightly acidic electrolyzed water pre-cooling, natural convection pre-cooling, fluid ice pre-cooling, cold water pre-cooling, vacuum pre-cooling, and strong wind pre-cooling, respectively.

reaching 2.50 U/g. At the end of the storage period, the APX activity of the VPC-treated group was significantly higher than those of the other groups ($p < 0.05$). From Fig. 6(C), it can be seen that the peak CAT activity of bok choi in the pre-cooling group occurs later than that of the RT group, indicating that pre-cooling and cold storage treatment can enhance CAT activity and effectively delay the peak CAT activity in bok choi. In the later stages of storage, the CAT activity of bok choi in the VPC-treated group and SWPC-treated group decreased slowly and remained at a relatively high level, with values of 312.06 U/g and 282.63 U/g respectively, significantly higher than those of the other treatment groups ($p < 0.05$). This indicates that VPC treatment can affect the accumulation of reactive oxygen species (ROS) by increasing the activity of non-enzymatic antioxidants and antioxidant enzymes, and minimizing lipid peroxidation damage to cell membranes, thereby delaying vegetable ripening and senescence (Wang et al., 2022). These research findings were consistent with Juan et al.'s (Juan et al., 2020) study on Chinese cabbage and Liu et al.'s (Huan et al., 2016) study on *Aralia elata*, indicating that VPC treatment can enhance CAT activity in different plants. This further supports the regulatory role of VPC treatment on the antioxidant system, thereby delaying cell aging and alleviating lipid peroxidation damage to cells. These research findings provide strong evidence for the application of VPC treatment in preserving agricultural products and extending shelf life.

3.7. PPO, pod

PPO is a common metal enzyme in plants, with a catalytic site containing Cu^{2+}. When crops age or tissues are damaged, PPO located in the plastids is activated. It reacts with polyphenolic substances in the vacuoles to produce brown pigments, leading to plant browning and deteriorating the sensory quality of the crops in an unacceptable direction (Lante, Tinello, & Nicoletto, 2016). Fig. 6(D) presents the trend of PPO activity in small bok choi during the refrigeration period after being subjected to different pre-cooling treatments. It can be observed that PPO activity initially increased and then decreased. After 15 days of storage, the PPO activity in the VPC-treated group was significantly lower than those of other groups ($P < 0.05$). This indicates that VPC treatment can effectively inhibit the increase in PPO activity in bok choi, thereby suppressing browning and ensuring the quality of bok choi.

POD is widely present in different tissues of plants. As a highly active adaptive enzyme, it can reflect the characteristics of plant growth and development, internal metabolic status, and adaptability to the external environment. POD is mainly expressed in response to stress or during the late stages of aging. It participates in the generation of ROS, and chlorophyll degradation, and can trigger membrane lipid peroxidation, resulting in damage effects. It is a product of plant aging to a certain stage and can serve as an indicator of plant senescence (Balwinder et al., 2018). As shown in Fig. 6(E), during storage, the POD activity of each group shows an initial increasing trend. This is because bok choi matured and aged, and the accumulation of reactive oxygen species stimulated the increase in POD activity. During storage, there were significant differences in POD activity among the different treatment groups. The POD activity in the FIPC treatment group was significantly higher than those of other groups ($p < 0.05$). This is mainly because the bok choi in the FIPC pre-cooled treatment group had a higher respiration rate and more vigorous metabolism during storage, leading to the accumulation of H_2O_2 and oxygen free radicals, which caused membrane lipid peroxidation and accelerated the aging of vegetables and fruits. The NCPC treatment had a low pre-cooling cooling rate, and during the pre-cooling stage, there was vigorous respiration, leading to the accumulation of harmful substances such as free radicals generated from the metabolism of energy and nutrients, which promoted product aging. The POD activity of the VPC treatment group remained at a relatively low level, and on the 15th day of storage, the activity was significantly lower than that of the other groups ($p < 0.05$). This may be because the VPC treatment effectively reduced the respiration rate and energy metabolism of vegetables and fruits, reducing the accumulation of reactive oxygen species during pre-cooling and the consumption of energy substances such as ATP. This enhanced the stress resistance of bok choi and delayed tissue aging, indicating that the VPC treatment was the best way to inhibit oxidative aging of bok choi during pre-cooling. This was similar to the results of Weiqi et al.'s (Weiqi et al., 2019) study on VPC treatment of broccoli.

4. Conclusion

FIPC treatment in the pre-cooling group resulted in a rapid decrease in the hardness of bok choi, leading to issues such as rotting and wilting. This was due to the direct contact with ice water during pre-cooling, causing cold damage to bok choi and disrupting its tissue structure. Furthermore, it was prone to microbial contamination during the later stages of storage, which negatively impacted the quality and storage period. The EWPC treatment and CWPC treatment had moderate effects in water pre-cooling. Although the EWPC treatment had some bactericidal effects, water pre-cooling left residual moisture on the surface of fruits and vegetables, making them susceptible to bacterial growth during storage. Natural convection pre-cooling leads to higher weight loss and lower quality during storage due to longer cooling times, strong respiration as well as transpiration consumption for a certain period of time after harvest. The SWPC treatment, however, showed positive results, maintaining good quality during the early stages of storage.

In contrast, VPC treatment can best maintain bok choi's high chlorophyll content, and moisture content. It helps to preserve the high chlorophyll content and antioxidant enzyme activity of bok choi, delaying browning significantly. It also reduces weight loss rate, respiration intensity, and relative electrolyte leakage, increases TVC, slows down the decrease of VC, soluble sugars, TA, and TP, and inhibits the formation of MDA. It enhances antioxidant enzyme activity leading to high ROS scavenging potential, thereby slowing down the aging process and maintaining fruit quality attributes.

Therefore, VPC treatment is the best post-harvest pre-cooling method for bok choi and some other leafy vegetables.

Funding

This work was supported by key projects in the field of agriculture by the Shanghai Municipal Commission of Science and Technology (23N31900100), and the Shanghai Professional Technology Service Platform on Cold Chain Equipment Performance and Energy Saving Evaluation (19DZ2284000). All authors have read and agreed to the published version of the manuscript.

Ethical Declaration

Before starting the sensory evaluation, all participants were informed about the experimental sample information, and the sensory evaluation was performed following the Helsinki Declaration. Due to the lack of a human ethics committee, this experiment was unable to obtain formal documentation procedures. However, during the research period, participation was not compulsory. All participants agreed to take part, understood the purpose, content, and risks of the experiment, and consented to the disclosure of the results of sensory evaluations. Any sensory assessors who feel uncomfortable with this work can choose to withdraw.

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

CRediT authorship contribution statement

Shaoyu Tao: Writing – original draft, Software, Investigation. **Jinfeng Wang:** Writing – review & editing. **Jing Xie:** Supervision, Project administration, Methodology, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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