REGULATION OF POST-HARVEST SUPEROXIDE DISMUTASE AND CATALASE ACTIVITIES IN CUCUMBER AND THEIR ROLE IN DELAYING AGING

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Abstract. In order to explore the role of superoxide dismutase and catalase activities in inhibiting the aging process of cucumber (*Cucumis sativus* L.), quantitative spectrophotometric analysis was used to monitor the activities of superoxide dismutase and catalase during storage of cucumber. Samples of refrigerated cucumbers were taken out for analysis at specified time points. The results of cucumber activity test showed that superoxide dismutase (superoxide dismutase, SOD) activity decreased from 482.76 U/g at the beginning to 282.58 U/g at 30 days, which decreased by 200.18 U/g, that is, an average daily decrease of about 6.67 U/g. The antioxidant capacity and soluble protein content of cucumber also increased, reaching 448.5 μmol Trolox/g on day 30, an increase of 85.6% over the initial level. These results indicate that the activities of superoxide dismutase and catalase in cucumber are regulated under cold storage conditions, and the two enzymes are important for maintaining physiological stability and delaying senescence in cucumber.
Keywords: *relative conductivity, gene expression, aging mechanism, refrigeration conditions, growth and development*

Introduction

In the agricultural industry, cucumber (*Cucumis sativus* L.), as a widely cultivated vegetable, its preservation and storage have always been important links in the industry chain (Torabi et al., 2022). Due to the complex physiological and biochemical processes that cucumbers undergo after harvesting, these processes directly affect the shelf life and quality of cucumbers. Especially superoxide dismutase (superoxide dismutase, SOD) and catalase (catalase, CAT), two key antioxidant enzymes, play crucial role in the physiological changes of cucumber after harvesting (Tashla et al., 2021). They can eliminate reactive oxygen species and reduce the damage of oxidative stress to cucumber cells, thus extending the shelf life of cucumbers (Abo et al., 2021). However, how to effectively regulate the activity of these two enzymes to maximize the delay of cucumber aging is always a research hotspot in preserving agricultural products (Toppo et al., 2023). Therefore, many scholars have studied the correlation between enzymes and cucumber aging. For example, Brengi et al. (2022) studied the effects of melatonin on SOD and CAT in cucumber plants and analyzed the mechanisms by which SOD and CAT affected cucumber senescence. In addition, Amin et al. also analyzed the SOD and CAT activities of cucumbers under low temperature and high humidity stress and studied the correlation between SOD and CAT activities and cucumber aging mechanisms (Amin et al., 2021). Nedved et al. (2022) also studied the activities of SOD and CAT in cucumber seedlings

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under salt stress conditions. SOD and CAT activities were related to cucumber aging. In this context, research was conducted on the regulation of post-harvest SOD and CAT activities in cucumber. This not only has theoretical value, but also practical guidance significance (Shin et al., 2022).

Although many studies have explored the role of SOD and CAT in cucumber preservation, there are still some key knowledge gaps that need to be filled. First of all, although SOD and CAT are known to play an important role in clearing reactive oxygen species and reducing oxidative stress, there is still insufficient research on how to specifically regulate the activity of these two enzymes to maximize the delay of the aging process in cucumber. In particular, the dynamic changes of SOD and CAT activities under different refrigeration conditions and the specific relationship between them and the aging mechanism of cucumber have not been fully revealed. Secondly, most of the existing studies focus on the effect of a single storage condition on the enzyme activity of cucumber, and lack of systematic comparison of the combined effect of different storage conditions (such as temperature, humidity, light, etc.). In addition, although some studies have shown that the changes in the expression levels of SOD and CAT genes are closely related to the enzyme activity, the specific regulatory mechanisms of these genes under cold storage and their roles in the aging process of cucumber are not fully understood. In particular, there are few studies on the differences of enzyme activity and gene expression in different cucumber varieties during cold storage. Therefore, this study designed an experiment to explore the effects of cold storage conditions on the activities of SOD and CAT in cucumber, and further analyze the role of these two enzymes in delaying senescence. The purpose of this study is to optimize the storage conditions and regulate the activity of SOD and CAT in cucumber, so as to bring considerable economic benefits to the agricultural products industry and meet consumers' demand for fresh and high-quality agricultural products. The innovation of research is reflected in three aspects. Firstly, high-precision measurement techniques such as NBT method and UV spectrophotometry are used to ensure the accuracy of enzyme activity measurement. Secondly, by observing the cell structure through transmission electron microscopy, the impact of enzyme activity changes on cucumber cell structure can be visually observed. Finally, advanced statistical software is used in data analysis to ensure the reliability and accuracy of the research results. This study not only provides a new theoretical basis for cucumber preservation technology, but also provides methodological references for other research on agricultural product storage and preservation.

Materials and methods

Material sources

To ensure the accuracy and reliability of this study, the cucumber samples selected were all from farmland with consistent planting and harvesting conditions. These farmland followed standardized agricultural management practices, including soil preparation, planting techniques, irrigation, and fertilization, to ensure the healthy growth of cucumber plants and uniform ripening of fruits (Lee et al., 2022). When harvesting, cucumbers with good growth, no pests or diseases, and similar appearance were selected as experimental materials to minimize the impact of individual differences on the experimental results. After harvesting, cucumber samples were immediately preprocessed, including cleaning, grading, and preliminary quality evaluation, to ensure that the samples were consistent and provide a reliable basis for subsequent experiments

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(Kumari et al., 2022). In this study, in order to ensure the reliability and repeatability of the experimental results, a total of 100 cucumbers with good growth, no pests and diseases and similar appearance were selected from the same farmland as the experimental materials. The cucumbers were evenly distributed to different experimental groups, each of which contained 10 cucumbers, to ensure statistical significance and confidence in the results. At each time point (such as day 0, day 10, day 20, day 30, etc.), 2 cucumbers were randomly taken from each experimental group for analysis to ensure the randomness and representativeness of the sample.

Experimental design

The experimental design aims to evaluate the effects of refrigeration conditions on the activities of SOD and CAT in cucumbers and the effects of SOD and CAT activities on cucumber aging process. Firstly, multiple refrigeration conditions, including different temperatures, humidity, and storage times were set, and the optimal refrigeration conditions were obtained through comparative experiments. Subsequently, under the optimal refrigeration conditions, the changes in SOD and CAT activities of cucumber after refrigeration treatment were analyzed. In addition, this study also set up indicators for measuring the cucumber aging under different SOD and CAT activities, hoping to analyze the impact of SOD and CAT activities on cucumber aging through this experiment.

Experimental analysis equipment

In this experiment, a variety of high-precision instruments were used to ensure the accuracy and reliability of the experimental data. The following are the types and manufacturers of the main analytical equipment:

UV Spectrophotometer: For the determination of CAT activity, the Shimadzu UV-1800 UV spectrophotometer is used (manufacturer: Shimadzu Production Company, Japan). The device is highly sensitive and stable and is able to accurately measure the absorbance change of the sample at 240 nm to calculate the activity of CAT.

Spectrophotometer: For the determination of SOD activity by the Nitroblue tetrazolium method (Nitroblue tetrazolium method, NBT) method, the BioTek Synergy H1 multifunctional enzyme labeler (manufacturer: BioTek Instruments, Inc., USA) was used. The device is equipped with an advanced optical system that can automatically read and process the absorbance data of multiple samples at 560 nm, improving experimental efficiency.

Transmission electron microscope: Used to observe the cell structure of cucumber, using JEOL JEM-1400 transmission electron microscope (manufacturer: Nihon Electronics Co., LTD., Japan). The microscope has high resolution and high magnification and can clearly show the subtle changes in cell wall, cell membrane, mitochondria, chloroplasts and other cell structures.

Quantitative Polymerase Chain Reaction (Polymerase Chain Reaction, PCR) instrument: Used to analyze SOD and CAT gene expression levels using the Applied Biosystems QuantStudio 7 Flex real-time PCR system (manufacturer: Thermo Fisher Technologies, USA). The device can monitor the fluorescence signal changes during the PCR reaction in real time, so as to accurately calculate gene expression.

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Experimental methods

Cucumber sample pre-treatment

The pre-treatment of cucumber samples includes steps such as cleaning, grading, pre cooling, slicing, disinfection, and drying (Wang et al., 2023). The purpose of these steps is to remove impurities from the surface of the sample, ensure consistency in sample size and appearance, reduce microbial activity, and prepare for subsequent enzyme activity measurements and refrigeration experiments. *Table 1* shows the details of cucumber sample pre-treatment (Byun et al., 2021).

Table 1. *Pre-treatment of cucumber samples*

Step No.	Process	Parameter settings	Detailed description
1	Ceaning	0.05% sodium hypochlorite solution	To remove surface contaminants and reduce microbial load.
2	Grading and selection	Length 15-20cm, diameter 3-5cm	Ensuring uniformity of samples by excluding undergrown and damaged specimens.
3	Pre-cooling	4°C, duration 2 hours	Lowering temperature to slow down the freshness decline rate.
4	Slicing to standard pieces	Thickness 5mm	Standardizing sample size for uniform handling in experiments.
5	Surface sterilization	70% ethanol solution, immersion for 1 minute	Further reducing microbial contamination risk and ensuring experimental safety.
6	Drying	Air drying at room temperature	Removing excess moisture to prevent water from affecting the experimental results.

In *Table 1*, pre-treatment starts from cleaning to remove surface impurities, and then ensures consistency in size and appearance of all samples through grading. Pre-cooling aims to slowly lower the temperature of cucumbers to reduce microbial activity and delay aging. Slicing and disinfection are further operations for preparing samples for subsequent experiments, while drying to remove surface moisture and preparing for refrigeration experiments. The cucumber samples are prepared to a suitable state. Each step is carefully designed to minimize the influence of external variables, ensuring the accuracy and reproducibility.

Refrigeration experiment

The preprocessed cucumber samples were stored under the designed refrigeration conditions. During storage, samples were collected regularly for enzyme activity measurement and analysis of other related indicators (Lu et al., 2021). *Table 2* shows the design parameters for the refrigeration experiment.

Table 2 shows the specific experimental parameters for different refrigeration experiments. Different groups' main differences are temperature and humidity. The optimal refrigeration environment was determined by measuring the SOD and CAD activities of cucumber under different experimental parameters and aging conditions mentioned above. Subsequently, various indicators of cucumber were tested in the optimal refrigeration environment to explore the impact of refrigeration conditions on changes in SOD and CAT activity.

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Table 2. *Refrigeration experiment design parameters*

Group	Temperature (°C)	Humidity (%)	Storage time (day)	Light conditions
1	8	85	30	Dark
2	8	80	30	Dark
3	8	90	30	Dark
4	6	85	30	Dark
5	6	80	30	Dark
6	6	90	30	Dark
7	10	85	30	Dark
8	10	80	30	Dark
9	10	90	30	Dark

Superoxide dismutase and catalase activity determination

NBT and UV spectrophotometry have the advantage of high accuracy, ensuring the accuracy of SOD and CAT activity measurements (Xu et al., 2021; Muhee et al., 2023). Therefore, NBT and UV spectrophotometry were used to determine the SOD and CAT activities in cucumber samples, respectively. *Table 3* provides detailed information on the measurement techniques used (Du et al., 2021; Szénási et al., 2023).

Table 3. *Detailed information on measurement techniques*

Technique	Target enzyme	Principle	Detection wavelength	Accuracy	Notes
Nitroblue tetrazolium (NBT) method	SOD	Indirectly measures SOD activity by monitoring the rate of NBT reduction.	560 nm	±2%	Avoid direct exposure to light.
Ultraviolet spectrophotometry	CAT	Measures CAT activity by quantifying the consumption of H_2O_2.	240 nm	±1.5%	Reaction temperature controlled.
Bradford protein assay	Total protein	Uses coomassie brilliant blue G-250 binding to quantify protein concentration.	595 nm	±2%	To normalize enzyme activity data.
Amino acid radical method	Antioxidant capacity	Assesses total antioxidant capacity by measuring samples ability to scavenge specific radicals.	412 nm	±3%	Supplementary indicator for antioxidant defense system evaluation.

Table 3 not only involves direct determination of SOD and CAT activity, but also includes measurement of total protein concentration and evaluation of antioxidant capacity. This provides specific detection of changes in the antioxidant defense system of cucumbers during storage. Meanwhile, by controlling experimental conditions such as avoiding direct sunlight and controlling reaction temperature, the reliability of the measurement results is further ensured. By selecting and controlling experimental

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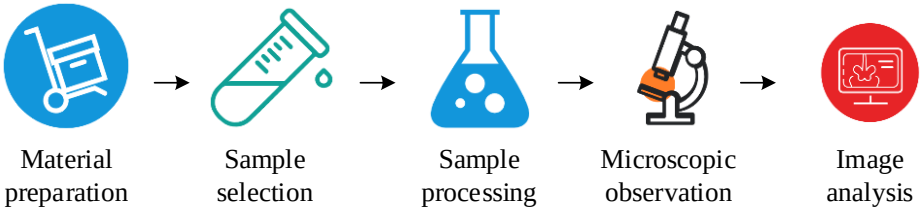
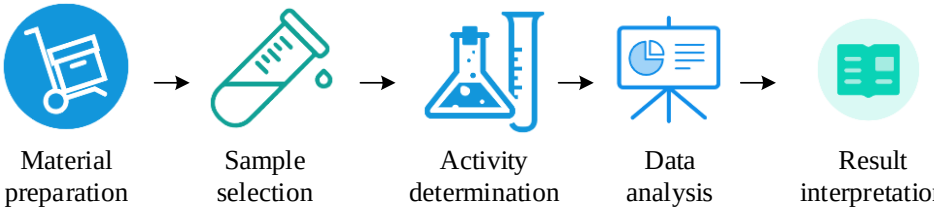
conditions, the measurement technique can accurately reflect the changes in antioxidant enzyme activity in cucumber after harvesting.

Observation experiment on cucumber cell structure

To observe the cell structure of cucumber under different SOD and CAT activities and analyze the relationship between SOD and CAT activities and cell aging markers, the experiment in *Figure 1* was conducted.



(a) Experimental procedure for observation of cucumber cell structure



(b) Experimental procedure for correlation analysis of enzyme activity and cell senescence markers

Figure 1. *Experimental procedure of cucumber cell structure observation and enzyme activity correlation analysis*

Figure 1 (a) shows the experimental steps for observing the cell structure of cucumber under different enzyme activity conditions. Firstly, it should prepare transmission electron microscopy, cucumber samples (to ensure that the samples have varying SOD and CAT activity), necessary microscopy tools, and reagents. Afterwards, tissue samples with different enzyme activities were taken, and cucumber tissue was cut into thin sections suitable for transmission electron microscopy observation, and necessary fixation and staining treatments were performed. Subsequently, the ultrastructure of cucumber cells under different SOD and CAT activities was observed and recorded under a transmission electron microscope. Special attention should be paid to the differences in the structure of cell walls, cell membranes, organelles, and other structures. Finally, the obtained microscopic images are processed and analyzed to compare the differences in cell structure under different activity conditions. *Figure 1 (b)* shows the experimental steps for analyzing the correlation between enzyme activity and cellular aging markers. Firstly, it should prepare cell aging marker detection reagents and instruments, cucumber samples (ensuring that the samples have different levels of SOD and CAT activity), and data statistics and analysis software. Afterwards, tissue samples with different enzyme activities were taken, and the SOD and CAT activities of each sample were measured and recorded. Subsequently, specific reagents and instruments were used to detect the expression levels of cellular aging markers. Finally, Spearman correlation analysis was used to explore the relationship between SOD activity, CAT activity, and cellular aging markers. In this study, the ultrastructure of cucumber cells under different SOD and CAT

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activities was observed by TEM, focusing on key cellular organs such as cell membrane, cell wall, mitochondria and chloroplasts in cucumber leaf cells. In order to quantify the characteristics of these cellular organs, the following quantitative indicators and methods were developed: for the cell membrane, its integrity and thickness were assessed by observing the continuity and recording the fractures and holes, while the thickness was measured at multiple points on the TEM image and the average value was calculated; For the cell wall, it is necessary to quantify its thickness and observe the tightness and fracture of the fiber arrangement. Mitochondria need to count the number of mitochondria in a specific area, assess their morphology, and count the number of cristae; For chloroplasts, it is also necessary to count their numbers and assess their morphological integrity and the order of the thylakoid stack. See *Table 4* for details.

Table 4. *Quantitative indicators of the characteristics of different cellular organs*

Cellular organ	Quantitative index	Measurements/observations
Cell membrane	Integrality	Continuous without fracture (yes/no)
	Thickness (nm)	Mean value
Cell wall	Thickness (nm)	Mean value
	Fiber arrangement	Whether tightly packed, no fracture
Mitochondria	Quantity (per view)	Mean value
	Form	Normal/swollen/deformed
	Number of cristae (per mitochondrial)	Mean value
Chloroplast	Quantity (per view)	Mean value
	Form	Integrity/degradation
	Thylakoid stack condition	Orderly stack

In addition, all "cell aging markers" and quantification processes are shown in *Table 5*.

Table 5. *"Cell aging markers" and quantification processes*

Cell aging marker	Quantitative index	Quantization method
Relative conductivity	Cell membrane injury degree	Electrical conductivity values were measured. High electrical conductivity values indicated severe cell membrane damage and high cell aging.
Lipid peroxidation products (MDA) content	Degree of lipid peroxidation in cell membrane	The content of MDA in cell and cell membrane was determined by chemical method. High content indicated serious oxidative damage.
Membrane Integrity (TEM observation)	Transmission electron microscopy (TEM) was used to observe whether the cell membrane was continuous without fractures and holes	Cell membrane continuity was recorded, and the appearance of fractures and holes was regarded as impaired cell membrane integrity.
Mitochondrial Morphology (TEM observation)	Mitochondrial swelling and deformation	The morphology of mitochondria was observed and recorded. Swelling and deformation indicated damaged mitochondrial function and aging of cells.
Chloroplast morphology and thylakoid stacking	Chloroplast degradation, thylakoid stacking disorder	Chloroplast integrity and thylakoid stacking were observed and recorded. Degradation and disordered stacking indicated cell aging.

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Methods for detecting gene expression

In cucumber, the activities of CAT and SOD enzymes are regulated by several genes, which encode the catalytic subunits of enzymes involved in the synthesis and modification of enzymes. It usually includes the SOD gene family (including Cu/Zn-SOD, Mn-SOD and Fe-SOD, etc.), and the SOD isoenzymes encoded by these genes play a role in different parts of the cell, working together to remove superoxide anion free radicals. The CAT gene, which codes for catalase, usually has only one or a few copies in cucumbers. CAT enzyme can catalyze the decomposition of hydrogen peroxide into water and oxygen, thereby reducing the damage of oxidative stress on cells. The samples were quickly frozen with liquid nitrogen immediately after collection and stored in a -80°C refrigerator for later use. First, cucumber RNA should be extracted and purified. In this step, TRIzol reagent (Invitrogen, USA) should be used for RNA extraction according to the manufacturer's instructions. The extracted RNA is processed by DNase I (RNase-free, Takara, Japan) to remove genomic DNA contamination. The concentration and purity of RNA was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) to ensure that the A260/A280 ratio was between 1.8 and 2.0. The integrity of RNA was further verified by agarose gel electrophoresis.

Reverse transcription was then performed using the PrimeScript RT Reagent Kit with gDNA Eraser (Takara, Japan). In this step, the gDNA Eraser is first used to remove genomic DNA in RNA. cDNA is then synthesized using reverse transcriptase and random primers. gDNA removal is typically performed at 37°C for 15 minutes, followed by reverse transcriptase inactivation at 85°C for 5 seconds. Finally, quantitative PCR was performed. In this step, specific primers for SOD and CAT genes were designed according to the cucumber genome sequence. Primers for internal reference genes (such as β-actin or GAPDH) were also designed as controls. The primers were verified by NCBI Prime-BLAST. The reaction system was quantified by PCR using SYBR Premix Ex Taq II (Tli RNaseH Plus) (Takara, Japan). The reaction system includes cDNA template, positive and negative primers, SYBR Green dye and PCR buffer. Amplification was performed on Applied Biosystems QuantStudio 7 Flex real-time PCR system (Thermo Fisher Technologies, USA). The amplification procedure consists of predenaturation (95°C, 30 seconds) followed by 40 cycles of denaturation (95°C, 5 seconds) and annealing/extension (60°C, 30 seconds). 3 technique replicates were set for each sample. Finally, the relative expression of target genes relative to reference genes was calculated using ΔΔCt method. SPSS Statistics 22.0 software was used to conduct ANOVA and Duncans multiple comparison test to determine significant differences in gene expression between treatments.

Data analysis methods

The study used Microsoft Excel for data recording and organization. ANOVA and Duncan multiple comparison tests were conducted using SPSS Statistics 22.0 software to evaluate the effects of different storage conditions on SOD and CAT activities in cucumbers (Joshi et al., 2021; Wang et al., 2022; Matsuo et al., 2022). The graphical representation was done using OriginPro 9.4 to visually display the experimental results and trends (Matsuo et al., 2022). These software and strictly controlled experimental conditions aim to provide accurate quantitative data on changes in post harvest SOD and CAT activity of cucumbers, thereby supporting the agricultural product preservation.

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Results and analysis

Optimal refrigeration environment setting

To determine the optimal refrigeration environment for cucumbers, the SOD and CAT activities of cucumbers were tested under nine sets of refrigeration experimental environments in *Table 2*. By comparing the activities of SOD and CAT, the optimal refrigeration environment was determined by selecting the one with higher SOD and CAT activities. The comparison results of SOD activity and CAT activity in nine groups under refrigerated experimental environment are shown in *Table 6*.

Table 6. *Comparison results of Superoxide dismutase activity and catalase activity in nine groups under cold storage experimental environment*

Experimental group	Storage time (days)	SOD activity (U/g)	CAT activity (U/g)
Group 1	0	482.76	2.08
	10	405.65	7.65
	20	360.45	13.89
	30	282.58	2.136
Group 2	0	480.12	2.11
	10	395.78	6.54
	20	352.89	12.00
	30	278.90	1.98
Group 3	0	483.21	2.09
	10	400.11	7.00
	20	355.67	12.56
	30	280.12	2.05
Group 4	0	481.54	2.10
	10	398.76	7.89
	20	358.90	14.00
	30	285.45	2.22
Group 5	0	479.89	2.07
	10	396.54	6.98
	20	354.12	11.56
	30	281.23	2.01
Group 6	0	480.98	2.12
	10	401.23	8.23
	20	360.78	14.56
	30	284.12	2.25
Group 7	0	481.23	2.06
	10	397.89	6.78
	20	353.45	11.23
	30	279.87	1.95
Group 8	0	478.90	2.05
	10	395.45	6.32
	20	351.23	10.56
	30	277.65	1.89
Group 9	0	480.11	2.10
	10	399.87	7.56
	20	356.78	12.89
	30	281.56	2.11

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As can be seen from *Table 6*, among the four experimental environments, SOD activity in group 1 decreased relatively low. When the storage time was 30 days, SOD activity in group 1 was 353.5U/g, which was significantly better than that in groups 2, 3 and 4. In the last five experimental environments, the SOD activity of group 6 decreased relatively low. When the storage time was 30 days, the SOD activity of group 6 was 344.6U/g, which was better than that of groups 5, 7, 8 and 9. In summary, the overall level of SOD activity was higher in the first group. In addition, it was also found that in the first four experimental environments, the overall level of CAT activity in group 1 was higher than that in groups 2, 3 and 4, and its highest CAT activity was 16.3U/g. In the last five experimental environments, the overall level of CAT activity in group 5 was higher, and its highest CAT activity was 15.7U/g, which was better than that in groups 6, 7, 8 and 9. Overall, the overall level of CAT activity was higher in the experimental environment of group 1. In conclusion, SOD and CAT activities of cucumber were the best under the experimental conditions of group 1, so the cold storage environment was set as the experimental environment of group 1. In order to verify the significant effects of different cold storage conditions on SOD and CAT activities, the study conducted variance analysis, and the results of variance analysis were shown in *Table 7*. It can be seen from *Table 7* that SOD and CAT have significant differences under different refrigeration conditions (P<0.05), which further indicates that the experimental environment of group 1 is better.

Table 7. *Results of variance analysis*

Enzyme species	Source	Sum of squares	Degree of freedom	Mean square	F	P
SOD	Intergroup variation	103179.52	8	1321.12	18.65	<0.0001
	Intra-group variation	52130.13	81	668.148	/	/
	Total variation	173157.24	89	/	/	/
CAT	Intergroup variation	1018.15	8	127.931	15.32	<0.0001
	Intra-group variation	891.36	81	11.05	/	/
	Total variation	1913.57	89	/	/	/

Note: Inter-group variation refers to the difference between different refrigeration conditions, and intra-group variation refers to the error variation between different time points under the same refrigeration conditions. The P value was used to determine whether the difference between the groups was significant (P<0.05 meant significant difference)

Analysis of enzyme activity changes under optimal refrigeration conditions

Figure 2 shows the changes in SOD activity of two batches of cucumber samples under the optimal refrigeration condition.

In *Figure 2 (a)*, the SOD activity of cucumber sample A gradually decreased from 482.76 U/g on day 0 to 282.58 U/g on day 30, a decrease of 200.18 U/g. In *Figure 2 (b)*, under the same refrigeration conditions, the SOD activity of another batch of cucumber samples was 486.32 U/g, which decreased to 289.17 U/g on the 30th day, with a decrease of 36.42%. The above results indicated that as the refrigeration storage time of cucumbers increased, the decrease in SOD activity gradually decreased. Subsequently, the CAT activity of two batches of cucumber samples was measured. *Figure 3* shows the changes in CAT activity of two batches of cucumbers under refrigeration conditions.

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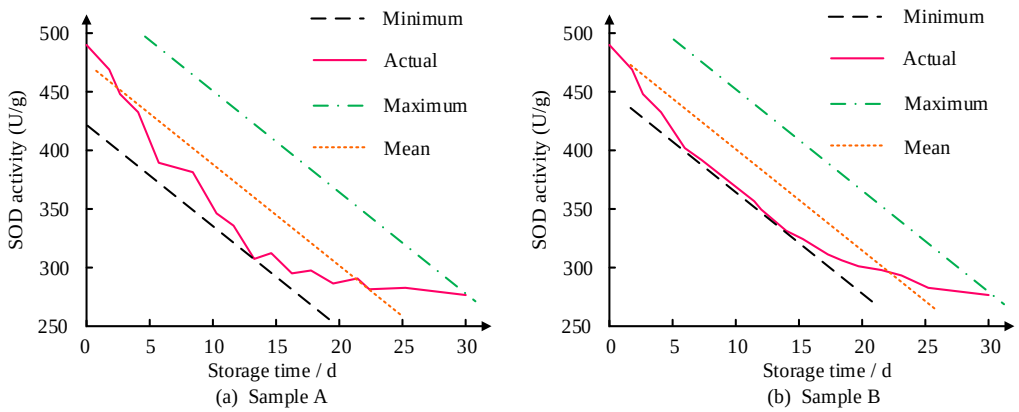

Figure 2. *Dynamic changes of Superoxide dismutase activity in cucumber under refrigeration conditions*

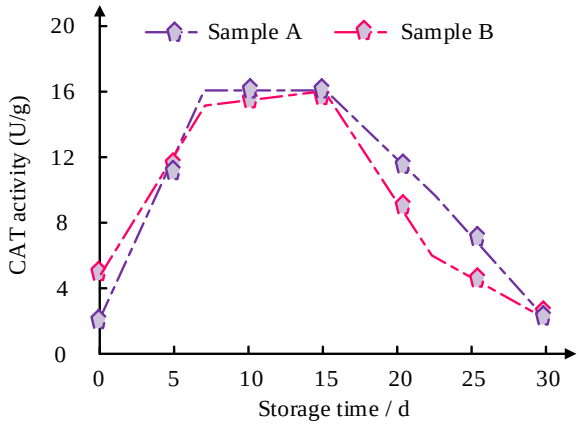

Figure 3. *Changes in catalase activity in cucumber under refrigeration conditions*

In *Figure 3*, the CAT activity of cucumber A experienced a significant increase in the early stage of storage, from 2.08 U/g on day 0 to 16.07 U/g on day 8, with an increase of nearly 7-fold. However, during the middle stage of storage, which was 9-15 days, the changes in CAT activity tended to stabilize. In the later stage of storage, after 15 days, CAT activity decreased, dropping to 2.136 U/g on the 30th day, which was 14.153 U/g lower than the 15th day. In addition, the overall changes in CAT activity of cucumber sample B were similar to those of cucumber sample A. The above results indicated that the CAT activity of cucumbers significantly increased in the early stage of refrigeration, then went through a stable period, and finally rapidly decreased in the later stage of cucumber storage. Finally, quantitative PCR was used to analyze the changes in SOD and CAT genes of cucumber antioxidant enzymes under refrigeration conditions in *Figure 4*.

Figure 4 (a) shows the gene expression rate of SOD gene in refrigeration. During the initial 0-10 days of refrigeration, there was a significant increase in SOD gene expression rate, which rapidly increased from 35.3% before refrigeration to 59.6% on the 10th day. Then it slowly increased to 78.5% on the 20th day and eventually dropped to 68.3% on the 30th day. *Figure 4 (b)* shows the changes in gene expression rate of CAT genes during refrigeration. The CAT gene expression rate also showed a certain degree of increase,

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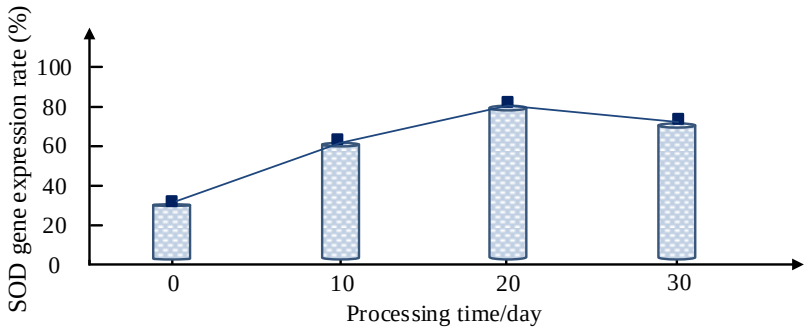
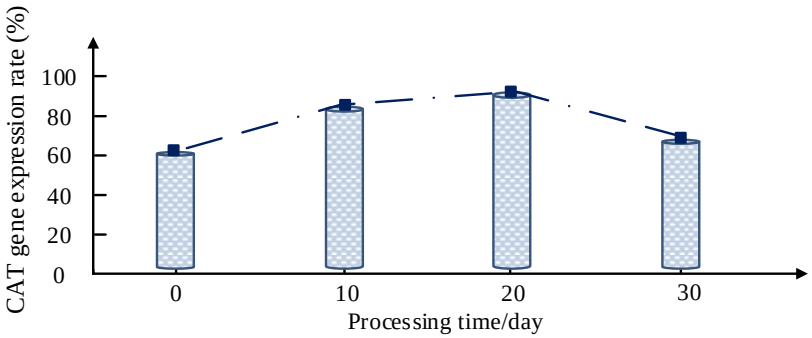
increasing from 60.3% at the beginning of refrigeration to 79.8% on the 10th day. Then it slowly rose to 86.5% on the 20th day, began to decrease after 20 days, and finally dropped to 60.2% on the 30th day. The above results indicated that by adjusting the expression of SOD and CAT genes, cucumbers enhanced their antioxidant defense ability and controlled intracellular oxidative pressure, avoiding cell damage.



(a) Changes in expression levels of SOD genes during refrigeration



(b) Changes in expression levels of CAT genes under the same refrigeration conditions

Figure 4. *Changes in the expression of cucumber antioxidant enzyme genes under refrigeration conditions*

Changes in total protein concentration and antioxidant capacity under refrigeration conditions

In addition, to better analyze the aging of cucumbers, the study tested the total protein concentration and antioxidant capacity of cucumbers under optimal refrigeration conditions. The total antioxidant capacity (total antioxidant capacity, TAC) was tested using the amino acid matrix method. *Figure 5* shows the test results.

Figure 5 (a) shows the changes in TAC of the first three cucumber samples under refrigeration conditions. The TAC of the three cucumber samples increased with the storage time. Cucumber sample 1 had the highest TAC at a storage time of 30 days, which was 448.5 μmol Trolox/g, higher than sample 2's 431.5 μmol Trolox/g and sample 3's 398.7 μmol Trolox/g. *Figure 5 (b)* shows the changes in TAC of the last three cucumber samples under refrigeration conditions. The TAC of the three cucumber samples increased with the storage time. Cucumber sample 4 had the highest TAC at a storage time of 30 days, at 449.3 μmol Trolox/g, which was higher than sample 5's 388.5 μmol Trolox/g and sample 6's 411.3 μmol Trolox/g. The above results indicated that during the early to middle stages of refrigeration, cucumbers responded to oxidative stress in the refrigeration environment by enhancing their TAC, which helped to delay aging and maintain the stability of cell structure and function.

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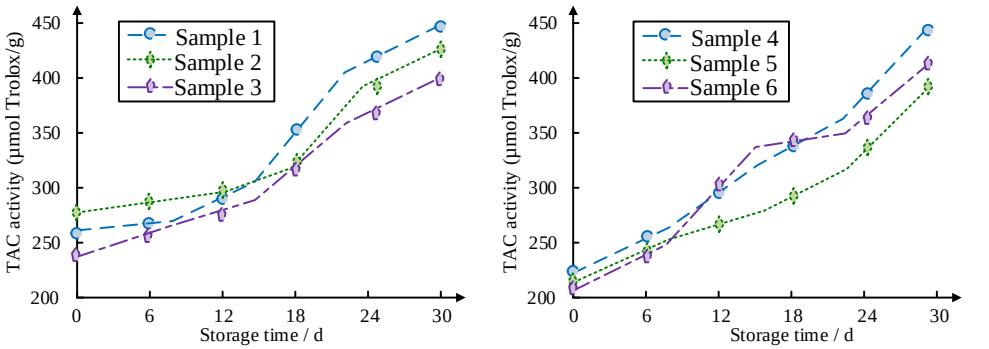

(a) Changes in TAC of the first three cucumber samples stored in cold storage for 0-30 days

(b) Changes in TAC after the three cucumber samples were stored in cold storage for 0-30 days

Figure 5. *Changes in cucumber total antioxidant capacity under refrigeration conditions*

Figure 6 shows the changes in malondialdehyde (malondialdehyde, MDA) in cucumber under refrigeration conditions. The increase of MDA is directly related to the aging mechanism of cucumber. MDA can inhibit the growth and development of cucumbers and affect the stability of cell membranes, especially under low temperature and high humidity refrigeration conditions.

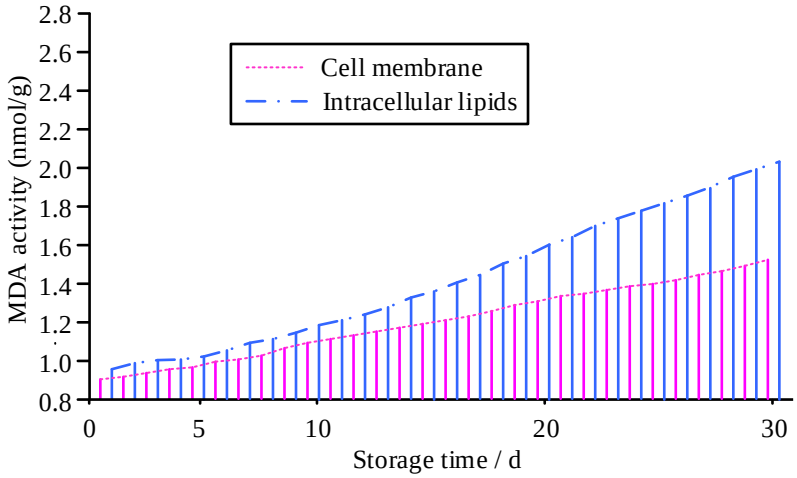

Figure 6. *Changes in malondialdehyde content in cucumber under refrigeration conditions*

In *Figure 6*, with the passage of time, the MDA content in cucumbers showed an upward trend. The intracellular MDA in cucumber cells increased from 0.97 nmol/L on the first day to 1.98 nmol/L on the 30th day. The MDA in the cucumber cell membrane increased from 0.92 nmol/L on the first day to 1.41 nmol/L on the 30th day. By comparing the MDA levels within cucumber cells and cell membranes, under refrigeration conditions, the growth rate of MDA in cucumber cells was higher than that in cucumber cell membranes. This change indicated that during refrigeration, the oxidative damage to cucumber cell membranes intensified, reflecting an increase in the activity of intracellular lipid peroxidation reactions. *Figure 7* shows the changes in soluble protein content in cucumber samples under refrigeration conditions.

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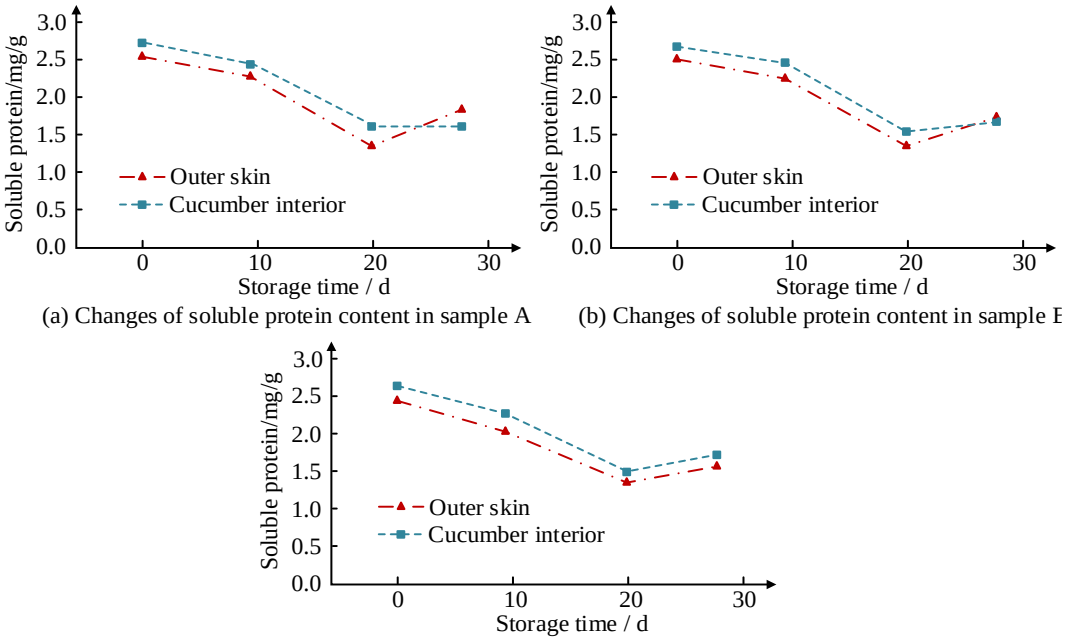

(a) Changes of soluble protein content in sample A (b) Changes of soluble protein content in sample B

(c) Changes of soluble protein content in sample C

Figure 7. *Changes of soluble protein content in different samples of cucumbers under cold storage*

Figures 7 (a), (b), and *(c)* show the changes in soluble protein content of cucumber samples A, B, and C, respectively. In *Figure 7 (a)*, on the 0th day, the soluble protein content in the flesh of cucumber sample A was 2.72 mg/g, which showed a trend of first decreasing and then increasing with the passage of time. On the 20th day, it dropped to its lowest value of 1.82 mg/g, and then slowly increased. By the 30th day, the soluble protein content was 1.85 mg/g. In addition, the trend of soluble protein content in the appearance of cucumber sample A also showed a decrease followed by an increase. The soluble protein content on day 0, day 20, and day 30 was 2.53 mg/g, 1.49 mg/g, and 2.08 mg/g, respectively. The overall trend of changes in *Figures 7 (a), (b),* and *(c)* was the same. The lowest soluble protein content in the flesh and exterior of cucumber sample B was 1.55 mg/g and 1.46 mg/g, respectively. The lowest soluble protein content in the flesh and exterior of cucumber sample C was 1.53 mg/g and 1.44 mg/g, respectively. The reason for the above changes in soluble protein content is that during the early stage of refrigeration, the metabolic activity and protein synthesis of cucumber cells are inhibited, leading to a gradual decrease in soluble protein content. In the later stage of storage, the activation of the response mechanism in cucumber leads to an increase in protein synthesis or a decrease in degradation, resulting in a slight increase in soluble protein content.

The effects of Superoxide dismutase and catalase activities on cucumber senescence

Figure 8 shows the relative conductivity of three cucumber samples under different SOD and CAT activities. The higher the relative conductivity, the higher the damage to the cell membrane, which in turn indicates a higher cucumber aging.

Figure 8 (a) shows the relative conductivity changes of three cucumber samples under different SOD activities. The increase in SOD activity resulted in a decrease in the relative

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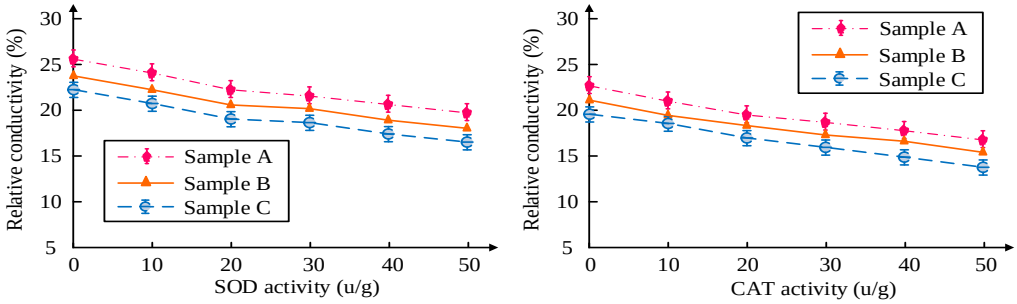
conductivity of all three cucumber samples. When the SOD activity was 50 u/g, the relative conductivity of cucumber samples A, B, and C was 20.8%, 19.7%, and 18.3%, respectively. *Figure 8 (b)* shows the relative conductivity changes of three cucumber samples under different CAT activities. With the increase of CAT activity, the relative conductivity of the three cucumber samples also showed a decreasing trend. When the CAT activity was 50 u/g, the relative conductivity was the lowest. At this time, the relative conductivity of cucumber samples A, B, and C were 18.5%, 17.7%, and 14.8%, respectively. The above results indicated that the activity of SOD and CAT in cucumbers was related to the trend of relative conductivity changes. Moreover, the stronger the SOD and CAT activities, the lower the relative conductivity of cucumbers. Subsequently, the cell structure of cucumber under high SOD and CAT activity and low SOD and CAT activity was magnified by 20000 times using a transmission electron microscope, and *Figure 9* was obtained.



(a) The relative conductivity of different cucumber samples under different SOD activity

(b) The relative conductivity of different cucumber samples under different CAT activity

Figure 8. *Curve plot of relative conductivity with refrigeration time*

From *Fig. 9*, in optimal cold storage (8°C, 85% humidity, dark storage), the microstructure of cucumber cells changed significantly after 30 days of storage. Specifically, the percentage of cell membrane integrity gradually decreased from 100% on day 0 to 60% on day 30, showing significant cell membrane damage. The thickness of the cell wall increased from an average of 350 nm on day 0 to 400 nm on day 30, with the fiber arrangement gradually becoming loose from tight, and even some fibers were broken. In terms of the number of mitochondria, an average of 15 mitochondria could be observed in each visual field at the beginning (day 0), and the morphology was normal and the number of cristae was clearly visible. By the middle stage (day 15), the number of mitochondria decreased to an average of 12 per visual field, and some mitochondria began to swell. By the end of the stage (day 30), the number of mitochondria has further decreased to an average of only eight per field of view, and most of the mitochondria have significantly deformed morphology, and the number of cristae has become blurred. The number of chloroplasts also experienced a significant decline, from an average of 20 intact chloroplasts per visual field at the beginning (day 0) to only 10 chloroplasts per visual field at the end (day 30), where most of the chloroplasts degraded and the thylakoid stack became disordered. These data not only strengthen the argument that storage conditions have a negative effect on the microstructure of cucumber cells, but also provide data support for further exploration and optimization of storage strategies to delay cucumber senescence. The above results indicated that high SOD and CAT activity effectively maintained the integrity of mitochondria and chloroplasts, thereby delaying

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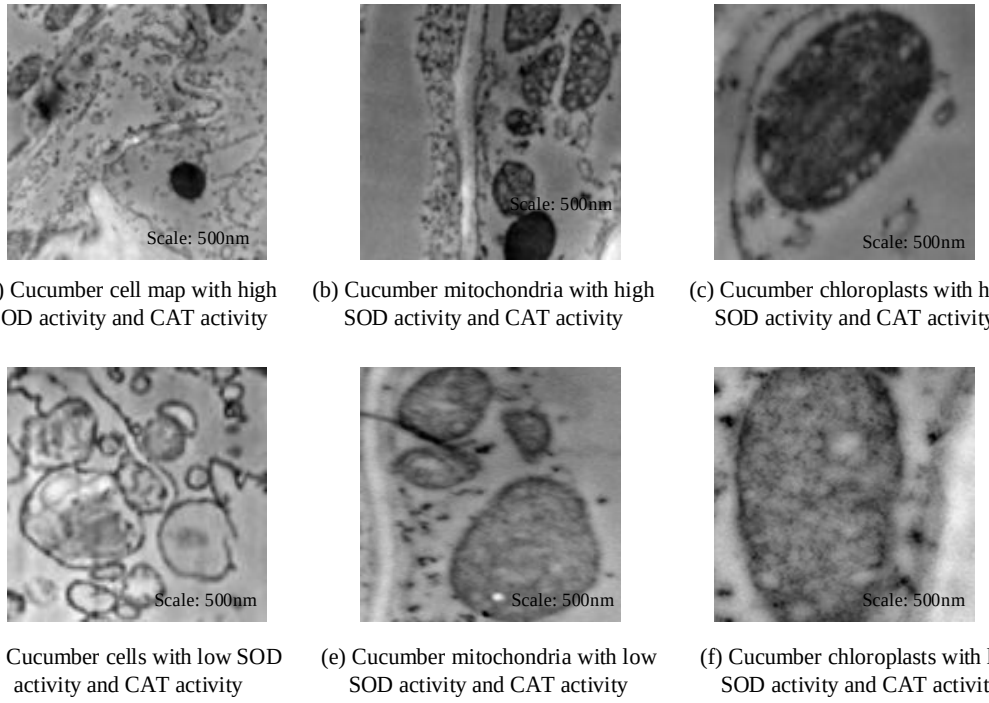
cucumber aging and maintaining better quality. Finally, Spearman was used to analyze the relationship between cucumber SOD activity and cellular senescence markers, as well as the relationship between CAT activity and cellular senescence markers. *Figure 10* shows the correlation analysis results.



(a) Cucumber cell map with high SOD activity and CAT activity

(b) Cucumber mitochondria with high SOD activity and CAT activity

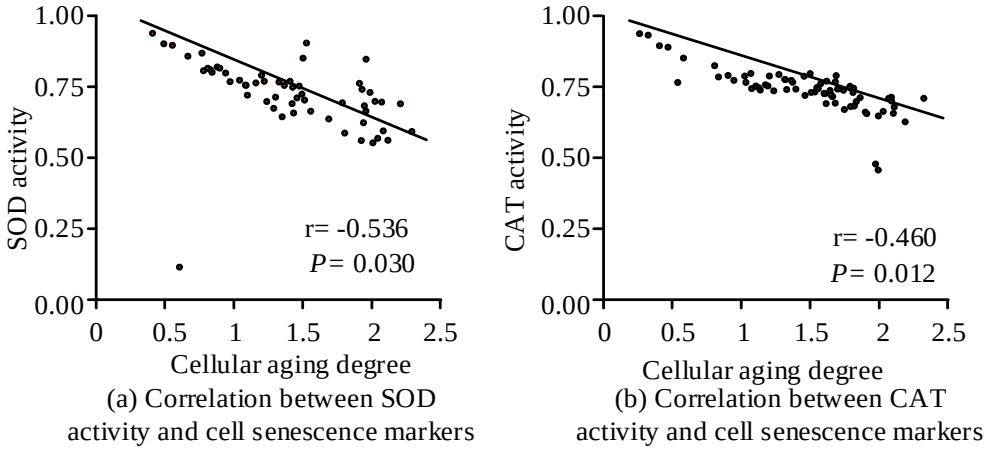
(c) Cucumber chloroplasts with high SOD activity and CAT activity

(d) Cucumber cells with low SOD activity and CAT activity

(e) Cucumber mitochondria with low SOD activity and CAT activity

(f) Cucumber chloroplasts with low SOD activity and CAT activity

Figure 9. *Detection of Superoxide dismutase and catalase activities in cucumber under refrigeration conditions using confocal laser scanning microscopy*



(a) Correlation between SOD activity and cell senescence markers

(b) Correlation between CAT activity and cell senescence markers

Figure 10. *Results of correlation analysis between Superoxide dismutase activity, catalase activity and cell senescence markers in cucumber*

Figure 10 (a) shows the correlation analysis results between cucumber SOD activity and cell aging markers. When the cell aging degree was 1, the corresponding value of SOD activity was 0.85. When the cell aging rate increased to 2, the corresponding value

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of SOD activity decreased to 0.55. In addition, the correlation coefficient between cucumber SOD activity and cell aging markers was -0.536. The above results indicated a negative correlation between cucumber SOD activity and cellular senescence markers. *Figure 10 (b)* shows the correlation analysis between CAT activity and cellular senescence markers in cucumber. When the cell aging degree was 1, the corresponding value of CAT activity was 0.88. When the cell aging rate increased to 2, the corresponding value of CAT activity decreased to 0.60. In addition, the correlation coefficient between cucumber CAT activity and cell aging markers was -0.460, indicating a negative correlation between cucumber SOD activity and cell aging markers. In summary, the enhancement of SOD and CAT activities enhanced the anti-aging properties of cucumbers, thereby increasing their storage time.

Discussion

Cucumber, as a common vegetable, has a refreshing taste and nutritional value, and is deeply loved by people (Matsuo et al., 2022). However, cucumbers are susceptible to various environmental factors during storage and preservation after harvesting, leading to a decline in quality and accelerated aging. To extend the shelf life and maintain the quality of cucumbers, researchers are committed to exploring various storage technologies and methods (Correa et al., 2021). During this process, SOD and CAT, two biological enzymes, have attracted widespread attention. These two enzymes play a role in clearing harmful substances such as free radicals and hydrogen peroxide in organisms and are of great significance in delaying cellular and tissue aging (Charles, 2024). In cucumber, SOD and CAT also play a role in resisting oxidative stress and protecting cells from damage (Shende et al., 2022). However, after harvesting cucumbers, they are detached from the nutrient supply of the mother plant, and their physiological and biochemical processes will undergo changes. This leads to a gradual decrease in the activity of SOD and CAT, which in turn affects the shelf life of cucumbers (Ussenov et al., 2022). Therefore, researchers begin to explore how to regulate the activity of these two enzymes in order to delay cucumber aging by increasing their activity. In this context, it is particularly important to study the regulation of post harvest SOD and CAT activities in cucumber and their role in delaying aging. Therefore, this study aims to gain a more accurate understanding of the relationship between these two enzymes and cucumber aging by conducting in-depth research on their activity changes under storage conditions such as refrigeration. This in turn provides theoretical basis and practical guidance for optimizing storage conditions and extending the freshness period of cucumbers. This can not only improve the economic benefits of agricultural products, but also meet the market's demand for fresh and high-quality agricultural products. It also helps to promote the development and innovation of agricultural product preservation technology.

This study delved into the changes in SOD and CAT activities in cucumbers under refrigeration conditions and their effects on cucumber aging. Under cold storage, the SOD activity of cucumber samples began from 482.76 U/g and gradually decreased to 282.58 U/g during the 30-day storage period. This reduction process shows a linear downward trend, with an average reduction of about 6.67 U/g per day. The decrease of SOD activity may be influenced by the slowing down of metabolic activity of cucumber cells and the down-regulation of antioxidant defense system. Different from SOD, the decreasing process of CAT activity showed a trend of first increasing and then decreasing. At the initial stage of cold storage (0-8 days), CAT activity increased significantly, which

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may be an adaptive response of cucumber cells to the low temperature environment, trying to cope with the oxidative stress caused by cold storage by increasing CAT activity. However, with the extension of storage time (9-30 days), CAT activity gradually decreased to 2.136 U/g by day 30, significantly lower than its peak of 16.07 U/g. The reduction in CAT activity may reflect an overall decline in the antioxidant capacity of cucumber cells after prolonged refrigeration. This result was similar to the conclusion obtained by Ignatenko et al. (2021) on the enzyme activity of cucumbers. In addition, cucumbers enhanced their antioxidant defense ability by adjusting the expression of SOD and CAT genes. This finding was consistent with the research findings of Turan et al., indicating that cucumbers maintain intracellular homeostasis by adjusting the expression of related genes in response to environmental stress (Turan et al., 2022). Under refrigeration conditions, the TAC of cucumbers increased with increasing storage time, which may be an adaptive response made by cucumbers to resist oxidative stress in the refrigeration environment. Meanwhile, the MDA content in cucumbers was on the rise, reflecting the gradual intensification of oxidative damage to cucumber cell membranes during refrigeration. This result was similar to the research results of Turan et al. on the trend of changes in relevant indicators of cucumbers (Yang et al., 2023). Through transmission electron microscopy observation, the cell structure of cucumber under high SOD and CAT activity was more complete, and the morphology of mitochondria and chloroplasts was also more normal. This further confirmed the important role of SOD and CAT in protecting cucumber cells from oxidative damage. The correlation analysis results showed a negative correlation between SOD and CAT activities in cucumber and cellular aging markers. This indicated that the enhancement of SOD and CAT activities delayed the senescence of cucumbers, thereby improving their storage time. This research result was consistent with the study conclusion of Fu et al. (2023) on cucumber enzyme activity in 2023.

In summary, this study delved into the changes in SOD and CAT activities in cucumber under refrigeration conditions and their effects on cucumber aging. These results indicate that the activities of these two enzymes exhibit specific trends during refrigeration and are closely related to the anti-aging ability of cucumbers. By enhancing the activity of SOD and CAT, cucumber aging can be effectively delayed, maintaining its freshness and quality. This discovery provides new ideas for optimizing cucumber storage conditions and extending the shelf life. It is expected to improve the economic benefits of agricultural products, meet the market demand for high-quality agricultural products, and promote the progress of agricultural product preservation technology.

Conclusion

This study revealed the activity changes of SOD and CAT enzymes in cucumber after harvesting under refrigeration conditions and their important role in delaying aging through in-depth research. These experiments revealed that the SOD of cucumbers decreased from 482.76 U/g to 282.58 U/g. However, CAT activity gradually increased from 2.08 U/g to 16.07 U/g, and then decreased to 2.136 U/g. In addition, the total antioxidant capacity of cucumbers was significantly enhanced during refrigeration, which indirectly demonstrated the contribution of antioxidant enzymes such as SOD and CAT in scavenging free radicals and reducing oxidative stress, thus helping to delay aging. At the same time, the change of soluble protein content also reflects the physiological response of cucumber to environmental stress. Transmission electron microscopy showed

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that cucumber cells with high SOD and CAT activity had more complete structure, and the morphology of mitochondria and chloroplasts remained good, which further confirmed the key role of these two enzymes in protecting cells from oxidative damage and delaying aging. Finally, Spearman correlation analysis showed that there was a significant negative correlation between SOD and CAT activity and cell senescence markers, which directly supported the important role of these two enzymes in delaying senescence. This result indicated a significant negative correlation between cucumber SOD activity and CAT activity and cucumber cell senescence markers. In summary, this study confirmed the key roles of SOD and CAT in delaying aging. By regulating the activity of these two enzymes, the shelf life of cucumbers can be effectively extended and their economic value can be improved. This provides a new theoretical basis for the storage and preservation of cucumbers. Although this study has achieved certain results, there are still some shortcomings. Firstly, this experiment mainly focused on refrigeration conditions and did not involve the impact of other storage methods such as controlled atmosphere storage on enzyme activity. Secondly, this experiment did not conduct comparative studies on different varieties of cucumbers to explore the differences in enzyme activity between varieties. Further research is needed to investigate the changes in SOD and CAT activity in cucumbers under different storage methods and the physiological and biochemical differences among different varieties of cucumbers during storage. In the future, methods should be explored to improve SOD and CAT activities in cucumbers through genetic engineering to provide more possibilities for innovation in cucumber preservation technology.

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Author contribution. In this paper, regulation of post-harvest superoxide dismutase and catalase activities in Cucumber and their role in delaying aging were discussed. Yupeng Fang put forward the research experiment: the effects of refrigeration conditions on the activities of SOD and CAT in cucumbers were studied. Faxiang Jiang analyzed the data and helped with the constructive discussion. Yupeng Fang did the experiments, recorded data, and created manuscripts. All authors read and approved the final manuscript.

Disclosure of interest. The authors report no conflict of interest.

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