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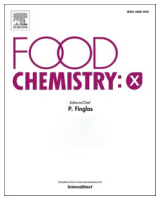

Prolong the shelf-life of the Pakchoi seedlings through the ammonium glycyrrhizinate

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ABSTRACT

Pakchoi seedlings (*Brassica chinensis L.*) is susceptible to damage and spoilage during harvest and transport, leading to significant quality deterioration and financial losses. This study explored the use of ammonium glycyrrhizinate (AG) to address these issues. AG self-assembles into macromolecules at room temperature, blocking stomata and regulating respiration rates in Pakchoi seedlings. Additionally, it disrupts bacterial cell biofilm and inhibits its synthesis. While AG has been used in medicine, its application in the food industry remains limited. The study found that incorporating AG in Pakchoi seedlings preserves water content and total soluble solids (TSS), while preventing declines in catalase (CAT), Vitamin C (VC), and chlorophyll during storage. AG also reduced malondialdehyde (MDA) levels and maintained peroxidase (POD) and superoxide dismutase (SOD) activities. At a concentration of 4.25 g L^{-1}, AG enhanced radical scavenging ability and extended the shelf life of Pakchoi seedlings by inhibiting bacteria and postponing senescence.

1. Introduction

In China, Pakchoi seedlings are a popular and widely cultivated green leafy vegetable, renowned for their high nutritional value, abundant dietary fiber, and a diverse array of minerals and multivitamins (Bang-di et al., 2021). However, Pakchoi seedlings possess large leaf surfaces and a high-water content, rendering them highly susceptible to wilting and yellowing post-harvest due to vigorous respiration and transpiration(Yang, Ren, & Zhu, 2022). This greatly influences the edible quality and market value of Pakchoi seedlings. Most leafy vegetables are sold in the form of unsorted and untreated raw produce, resulting in substantial waste due to the discarding of certain vegetables owing to untimely sales or inadequate storage and transportation(You, Zhou, Duan, Mao, & Li, 2022). Therefore, the developing net vegetable market requires suitable storage and preservation methods.

Glycyrrhizic acid (GA) and AG are both amphiphilic(Matsuoka, Miyajima, Ishida, Karasawa, & Yoshimura, 2016). Under suitable pH conditions, GA forms micellar structures, exhibiting similar aggregation behavior to bile salts (Polyakov, Magyar, & Kispert, 2013). AG consists of a hydrophobic triterpenoid glycoside unit, three hydrophilic carboxyl groups, and a free ammonium group (Scheme 1a). Similar to GA, AG can form worm-like nanostructures at pH 7.4, as observed by Liu et al. (2023)). The main distinction between GA and AG is the formation of a free ammonium group after salt formation(Matsuoka et al., 2016). Salt formation not only enhances the solubility of AG but also allows for spontaneous assembly into macromolecular aggregates (Scheme 1b, c).

AG has been recognized as "generally recognized as safe" by the FDA due to its antioxidant and antibacterial properties(Wu, Wang, Dou, & Gong, 2021). The spontaneous formation of macromolecular aggregates may play a crucial role in preserving vegetables. These dense micellar structures effectively cover the stomata of Pakchoi seedlings, preventing nutrient and water loss. AG demonstrates a remarkable free radical scavenging ability and effectively reduces intracellular reactive oxygen species (ROS) content. H_2O_2 is known to induce abnormal ROS content elevation within vegetable cells(Bonafe et al., 2022), whereas treatment with AG significantly lowers H_2O_2 content in vegetable cells. AG enhances cell clearance of free radicals, reducing oxidative stress damage. The antibacterial mechanism of AG was to inhibit bacterial cell biofilm synthesis and biofilm formation, reducing microbial growth, protecting vegetables from microbial influence, and extending their shelf life.

The objective of this study was to assess the preservative potential of AG on Chinese cabbage seedlings and investigate its impact on

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vegetables as well as bacteria. The application of an appropriate amount of AG solution at room temperature effectively extended the storage period of Chinese cabbage seedlings. Furthermore, AG is a convenient, cost-effective, and safe method, offering significant advantages over other preservation techniques.

2. Materials and methods

2.1. Materials and instruments

Commercially available fresh Pakchoi seedlings with relatively uniform and consistent specifications, from Shanghai Lingang Vegetable Marke; Sodium alginate (90%), oxalic acid, methanol, barium hydroxide, crystal violet solution, rhodamine, and phenolphthalein indicator, provided by Shanghai Macklin Reagent Co.; NBT and DAB reagent, provided by Nanjing built Co.; Anhydrous ethanol and anhydrous acetone were analytically pure, phosphate buffer (PBS, pH = 7.4, 0.1 M), VC content determination kit, free radical (DPPH, $O_2\cdot^-$, and ·OH) scavenging capacity kit, POD determination kit, SOD determination kit, chlorophyll content determination kit, and protein carbonyl content assay kit provided by Nanjing built Co; MDA content determination kit, and CAT content determination kit, provided by Beijing Solabao Co.; *E. coil*, *B.subtilis*, and *S.aureus* cultured in the laboratory of the College of Food, Shanghai Ocean University.

UV–Vis Spectrophotometer, UV-1900i, Suzhou Shimadzu Instruments Co.; High-speed freezing centrifuge, 5427R, Shanghai Eppendorf Co.; Enzyme labeller, FC type, Shanghai Thermo Fisher Instruments Co.; Electrothermal Constant Temperature Water Bath, HH-4, Shanghai Lichtenbosch Instrument Technology Co.; Moisture content analyser, HX-Q10, Shanghai HU Analytical Industry Co.; Constant Temperature Culture Oscillator, ZHWY-103B Shanghai Zhicheng Analytical Instrument Manufacturing Co.; Electrothermal constant temperature incubator, DHP-9162, Shanghai Yiheng Scientific Instrument Co.; pH meter, F2, Shanghai Mettler-Toledo Instrument Co.; Spectrophotometer, YS6010, Shenzhen SUNSHI Technology Co. Fourier Transform Infrared Spectrometer, Hoffen-20, Tianjin Jiaxinhai Machinery Equipment Co.

2.2. Methods

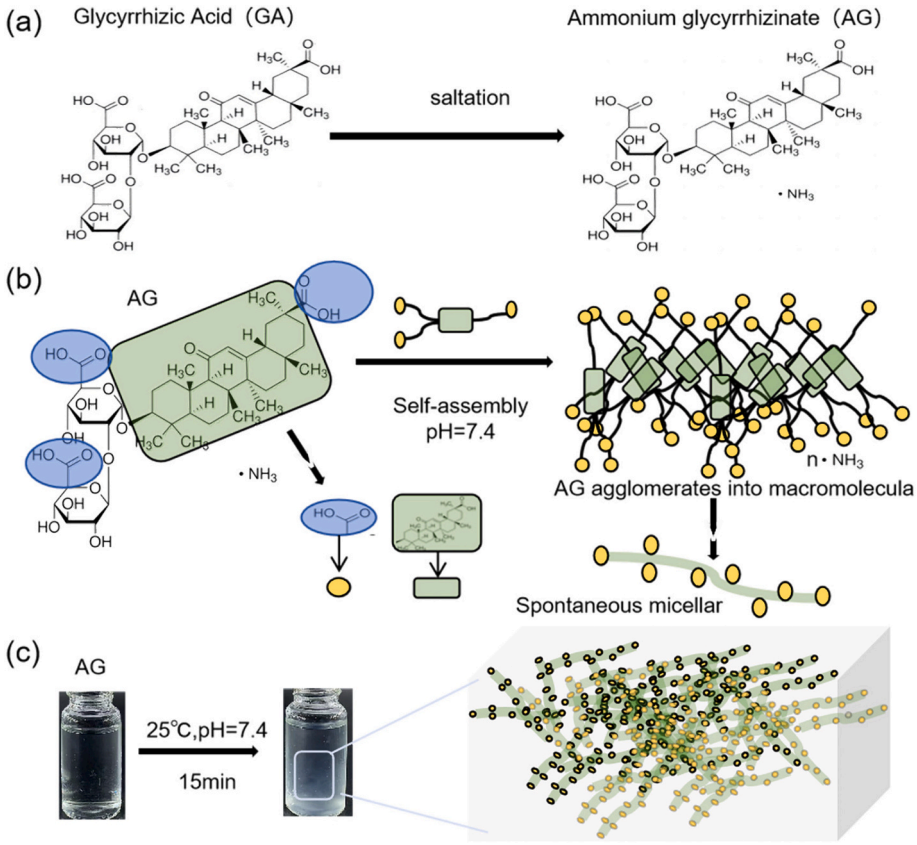
2.2.1. Sample treatment

Fresh Pakchoi seedlings with the same appearance were divided into five groups, and a CK was set up. Considering the physicochemical properties and practical application of AG, Pakchoi seedlings treated with different concentrations of AG (1.65, 2.50, 3.35, 4.25 g L^{-1}) were labeled as T1, T2, T3, and T4. Pakch oi seedlings were immersed with various concentrations of AG solution (25 °C, pH = 7.4) for 2 min before being placed in a ventilated area to dry rapidly and naturally before being stored in unsealed polyethylene preservation bags (the bags are perforated when used) at room temperature (25 °C, 40%–60% RH, 10 d). Three Pakchoi seedlings were taken from each group at two-day intervals to determine the indicators. To reduce measurement errors, we conducted identical measurements on all three Pakchoi seedlings and calculated the average. The detailed sampling locations for Pakchoi seedling leaves include the outer leaves, inner leaves, and root stems.

2.3. Determination of indicators

2.3.1. Apparent indicators

Sensory evaluation: Pakchoi seedlings are evaluated primarily based on their color, shape, scent, and texture. Weight-loss rate: Using the weighing method, the sample mass was weighed regularly and the weight-loss rate was calculated; Spoilage index is calculated using a rating method; Respiration rate: the resting method was used with



Scheme 1. Schematic representation of AG solution preparation. (a) Structural formula of GA and AG; (b) The structural formula and molecular stacking of AG; (c) Changes of cross-linking during the preparation of AG solution.

reference to Han et al. (2017), and the units were ng kg^{-1} s^{-1}; TSS content: to extract the juice, a portion of the sample is ground and filtered, and the soluble solids content of the sample is measured using a hand-held refractometer; Color difference: the color difference meter is used for determination.

The sensory evaluation panel consisted of 5 professionals in the food industry, who had received sensory evaluation training prior to the evaluation. The evaluators rated the sensory quality of the vegetables according to the standards presented in Table S1, and the average was calculated after multiple evaluations. Each item was rated out of a maximum of 10 points, and the scores were recorded. The final result was obtained by excluding the highest and lowest scores for each item and calculating the average.

Determination of color:

We measured the external leaves, internal leaves, and root stems of the Pakchoi seedlings. Each local area was measured three times to minimize errors.

Weight-loss rate:

Using the weighing method, the sample mass was weighed regularly and the weight-loss rate was calculated. For samples of the same treatment group, the measurement was repeated three times and the average value was taken to avoid errors.

$$W = (m_0 - m_1)/m_0{}^{*}100\% \quad (1)$$

where:

W—weight loss rate, %.
m_0—initial weight of Pakchoi seedling, Kg.
m_1—weight of Pakchoi seedling on the day of determination, Kg.

Spoilage index:

Spoilage index = ∑((decay level*number of vegetables at that level)/(highest decay level*total number of vegetables))*100%. The decay level is divided as follows: Vegetables are divided into four levels according to the size of the vegetable decay area: level zero for no decay; levelIfor decay areas <10% of the vegetable area; and level IIfor decay areas of 10% to 30% of the vegetable area. Level III is the decay area, which is >30% of the vegetable area.

Respiration rate:

$$V = [(V_1 - V_2)^{*}C^{*}44]/W^{*}t \quad (2)$$

where:

V_1—volume of oxalic acid solution used for titration of blank alkali solution, L.
V_2—volume of oxalic acid solution for titration of sample base solution, L.
C—molar concentration of oxalic acid, 0.1 mol L^{-1}.
44—molecular weight of CO_2, 10^{-3} Kg.
W—Weight of Pakchoi seedling sample, Kg.
t—The time of absorption of CO_2 by the alkali solution in the desiccator, s.

2.3.2. Optical characteristics and stability properties of AG

Preparation of a 4.25 g L^{-1} AG solution, stored at 25 °C, pH = 7.4, for 90 d. At the time points of 0 d, 30 d, 60 d, and 90 d, a small portion of the solution was sampled, and the absorbance values were measured and compared. The changes in absorbance at different time points were observed to evaluate the stability of the AG solution. Strict control of storage conditions was maintained throughout the experiment to ensure constant temperature and a stable pH of the AG solution. The same cuvette path length and solution volume were used to ensure the comparability of the results.

2.3.3. Antioxidant properties of AG solution

On 0 d, we immersed the first batch of fresh Pakchoi seedlings in a 4.25 g L^{-1} AG solution, and then quickly air-dried them in a well-ventilated area. The treated Pakchoi seedlings were then placed in unsealed polyethylene bags (perforated when used) and stored at room temperature (25 °C) for 10 d. On 0 and 10 d, we tested the treated seedlings for the following antioxidant activities: DPPH radical scavenging rate, ·OH scavenging rate, and $O_2{}^{\cdot -}$ scavenging rate. The changes in these indicators were calculated by subtracting the values obtained on 0 d from those obtained on 10 d to represent the changes in antioxidant performance. Additionally, after 30 d of storage (25 °C, pH = 7.4), we used the same method to measure the antioxidant activities of a second batch of fresh Pakchoi seedlings. We then compared the data from 10 d to 30 d. The changes in antioxidant performance were calculated by subtracting the values obtained on 10 d from those obtained on 30 d.

2.3.4. Antimicrobial properties of AG solution

We immersed the Pakchoi seedlings, which had been washed with distilled water, in solutions of different concentrations of AG soultion (0, 1.65, 2.50, 3.35, and 4.25 g L^{-1}). After 12 h, the juice was tapped out of the whole Pakchoi seedling preserved in a sterile sampling bag and diluted 10^{-5} times for plate coating, the total number of colonies on the Pakchoi seedlings was counted after 24 h.

E. coli, *S. aureus*, and *B. subtilis* were transferred into TSB test tube medium and each tube was filled with a certain amount of different concentrations of AG solution. The absorbance of the bacterial solution at 600 nm was measured every two hours and growth curves were plotted. The total number of colonies on Pakchoi seedling was determined by plate counting.

Different concentrations of AG solution were mixed with *E. coli*, *S. aureus*, and *B. subtilis* (Diluted 10^{-7} times), after 1 h, spread on plate, and plate counts were performed overnight. Create wells on the test plates containing different bacteria. Inject different concentrations of AG (100 μL) into the wells. For the control group, inject PBS. Upright the plates and incubate them in a 37 °C incubator. Observe the results and measure the inhibition circles after 18 h.

2.3.5. Bacterial oxidative stress testing

First, culture *E. coil*, *S. aureus*, and *B. subtilis* at 37 °C for 12 h until they reach the logarithmic growth phase. When the bacterial concentration reaches 10^7 CFU mL^{-1} (as observed using hemocytometer), take 1 mL of the bacterial suspension and centrifuge it. Remove the supernatant and resuspend the bacteria in 1 mL of PBS. Next, add different concentrations of AG solutions and incubate the mixtures. Use PBS as the control group. Finally, collect the bacterial cells and centrifuge them again (3000 rpm, 10 min, 4 °C). Wash the bacteria twice with PBS, resuspend them, and store for later analysis (The solution was placed in a biological refrigerator at 4 °C). Subsequently, measure the levels of bacterial ATP, MDA, SOD, and GSH-Px using test kits from Nanjing Jiancheng Bioengineering Institute.

2.3.6. Detection the carbonyl content of protein

E. coli, *S. aureus*, and *B. subtilis* were cultured at 37 °C for 4 h to reach the logarithmic growth phase, achieving a bacterial concentration of approximately 10^7 CFU mL^{-1}. Subsequently, 1 mL of bacterial suspension was centrifuged, and the bacteria were resuspended in 1 mL of PBS. Following this, varying concentrations of AG were added and incubated for 4 h. Finally, bacterial cells were collected by centrifugation at 3000 rpm for 10 min. After two washes with PBS, the bacterial were resuspended and stored. The protein carbonyl content was determined using the assay kit method by Nanjing built Co..

2.3.7. Bacterial cell biofilm test

The bacterial cell biofilm test procedure followed (Guo, Li, Wan, Zhou, Qin, & Gao, 2024), wherein 100 μL of AG was added to each well of a 96-well polystyrene plate to achieve final concentrations of 1.65,

2.50, 3.35, and 4.25 g L^{-1}. After incubating at 37 °C for 4 h, the culture medium was removed, and sterile PBS was slowly added to each well to remove residual suspended bacteria and other impurities. This process was repeated three times. Subsequently, the bacteria were fixed with methanol. After 15 min, the methanol was removed, and the plates were dried in a 40 °C vacuum oven. Then, 200 μL of 1% crystal violet solution was added for staining, and after 15 min, the staining solution was removed. The plates were washed with 200 μL of 1% crystal violet solution and then rinsed with 200 μL of PBS. Finally, 200 μL of 95% ethanol was added to dissolve the dye in each well. Absorbance was measured at a wavelength of 570 nm to indicate the extent of biofilm formation.

2.3.8. Biofilm potential of bacteria

The biofilm potential of bacteria testing procedure followed Yang's method (Yang et al., 2022), the biofilm potential of bacteria was determined using the rhodamine fluorescence method. A bacterial suspension of 2.3.5 was employed. Fluorescence of the mixture was detected at a wavelength of 530 nm upon excitation at 480 nm.

2.3.9. Enzyme index

Chlorophyll, POD, free radical (DPPH, $O_2\cdot^-$, and ·OH) scavenging ability, VC, SOD, for identification, follow the instructions provided by Nanjing Jiancheng Biotechnology Company. CAT and MDA, for identification, follow the instructions on the kit from Beijing Solabao Co..

2.3.10. Visualization of H_2O_2 and $O_2\cdot^-$ in situ accumulation

To observe the accumulation of H_2O_2 and $O_2\cdot^-$ in Pakchoi seedlings, we rinsed Pakchoi seedlings (outer leaves) with clean water and stained them histochemically with DAB (3, 3'diaminobenzidine) and NBT (nitro blue tetrazolium chloride), respectively. The coloring method is derived from(Sun et al., 2023).

Briefly, Pakchoi seedlings leaves (0 d, 5 d, 10 d) were soaked in DAB solution (1 g L^{-1}, pH = 5.5) for 4 h at 25 °C, and then soaked in 90% ethanol for 10 min in a water bath to remove chlorophyll. For the detection of $O_2\cdot^-$ accumulation, leaves were stained with NBT solution (0.5 mM, pH = 6) in the dark. Then observed the accumulation of DAB and NBT in leaves. During the ROS in situ staining process, T4 samples (from the outer leaves of Pakchoi seedlings) were taken, and the leaves of three different Pakchoi seedlings from the T4 group were tested.

2.3.11. Data processing

Each experiment was repeated three times, and the results were calculated separately and analyzed and processed using SPSS software, and the graphs were plotted using Origin 2021 software.

3. Results

3.1. Effect of AG treatment on shelf-life of Pakchoi seedlings

Fig. 1a illustrates the preservation and antibacterial mechanism of AG, which possesses the ability to self-aggregate into bundles of

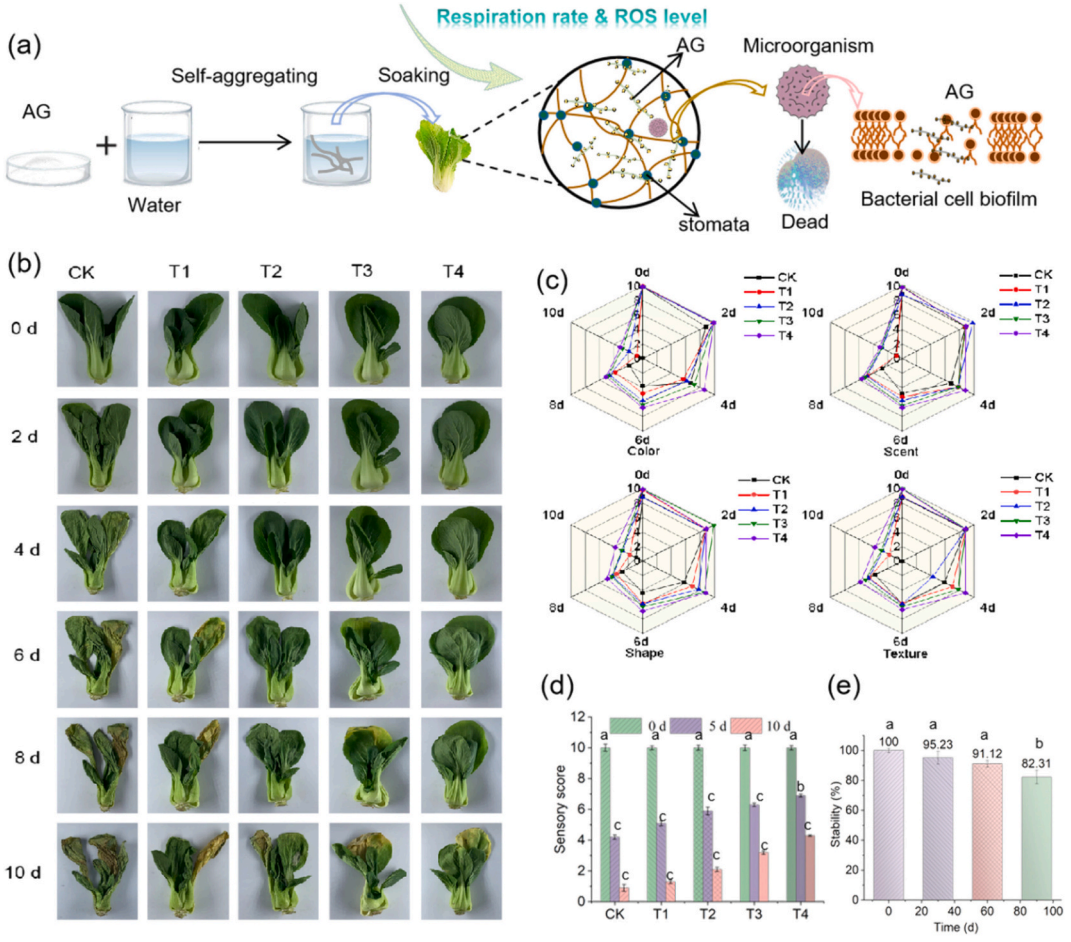


Fig. 1. Changes in Pakchoi seedlings treated with AG solution. (a) Mechanism of AG for preservation and antibacterial; (b) Changes in Pakchoi seedlings treated with different concentrations of AG solution during storage; (c) The Sensory changes in Color, Shape, Scent, and Texture on Pakchoi seedlings during storage; (d) The Sensory core in 0 d, 5 d, and 10 d; (e) Stability of 4.25 g L^{-1} AG solution (25 °C, pH = 7.4). Different present significant differences between groups ($p < 0.05$). (CK: control check, T1: 1.65 g L^{-1}, T2: 2.50 g L^{-1}, T3: 3.35 g L^{-1}, T4: 4.25 g L^{-1}).

macromolecules and effectively fill the leaf pores of Pakchoi seedlings. This process impacts the respiration rate, as depicted in Fig. 2c, while its antioxidant capacity regulates the level of ROS in Pakchoi seedlings, as shown in Fig. 3d, thereby maintaining their normal physiological state. Additionally, AG exhibits the capability to disrupt and hinder microbial cell biofilms, leading to a significant reduction in surface microorganisms on Pakchoi seedlings. The combined preservation and antimicrobial properties endow AG with the capacity to extend the shelf-life of Pakchoi seedlings. The effect of AG solution on the freshness of Pakchoi seedlings is shown in Fig. 1b, CK showed a decrease in shape and color from 4 d of storage, accompanied by wilting and browning, this is because Pakchoi seedlings have a high-water content after harvest, which makes them prone to yellowing and deterioration in the process of picking and storage, leading to a loss of their edible and nutritional value. After 8 d, despite some wilting and yellowing, T4 and T3 still showed good overall freshness and maintained their appearance even after 10 d. In Fig. 1c, after 2 d, the color, shape, scent, and texture scores of T1, T2, T3, and T4 were higher than those of CK. At 6 d, T3 and T4 had still higher scores than T1 and T2. After 8 d, T4's scores were higher than those of the other groups. In Fig. 1d, the total sensory score illustrates that T4 performs better in preserving the freshness of Pakchoi seedlings. Thus, AG could effectively prevent the sensory scores of Pakchoi seedlings from declining and maintained their freshness. Additionally, Fig. 1e demonstrates the stability of AG, ensuring its reliability and utility in preservation applications.

3.2. *Effect of AG treatment on physiological and biochemical indices of Pakchoi seedlings*

Fruit and vegetables lose water and weight due to respiration and transpiration after being harvested. The surface of fruit and vegetables will wrinkle and lose their fresh state once the weight-loss rate reaches a certain level(Zalewska, Marcinkowska-Lesiak, & Onopiuk, 2022). The weight-loss rates of all sample groups rose with longer storage times, as shown in Fig. 2a, but the treated group's weight-loss rates were consistently lower than CK's. At 4 d, T1, T2, T3, and T4 all showed lower weight losses than CK, suggesting that AG effectively inhibited weight loss in Pakchoi seedlings. At 6 d, CK's weight-loss rate was 6.32%, with T3 and T4 showing the lowest weight losses, at 3.86% and 3.55%, respectively. At 10 d, the weight-loss rate was 9.56% for CK. This suggests that the AG solution could prevent the increase in Pakchoi seedlings' weight-loss rates and preserve their storage quality.

The TSS content is an important quality indicator of fruit and vegetables(Alonso-Salinas et al., 2022). After 4 d, the TSS content of the different treatment groups varied, with 2.87% and 2.97% for T3 and T4, and 2.79% and 2.85% for T1 and T2, respectively. After 6 d of storage, the TSS content of Pakchoi seedlings in all treatment groups was higher than that of the CK (Fig. 2b). The magnitude of the respiration rate can reflect the strength of metabolic activity in vegetables(Y. Zhao, Nian, Wang, & Yang, 2022). When Pakchoi seedlings wilt, there is often an increase in the respiration rate, which is generally believed to be due to the lower water content of the leaves; The obstruction of photosynthetic product transport from the leaves results in an escalation of respiratory

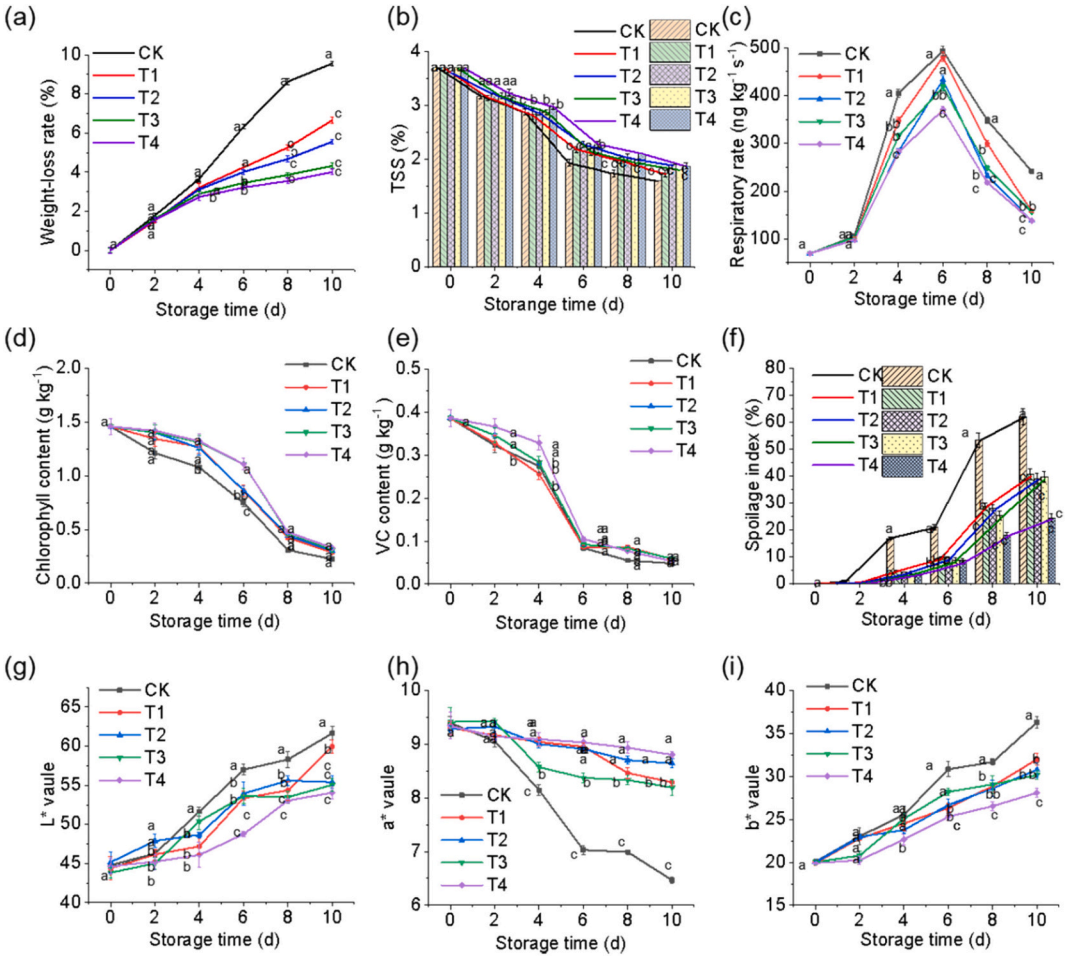


Fig. 2. The effect of AG treatment on Pakchoi seedlings during storage. (a) weight-loss rate; (b) TSS; (c) Respiration rate; (d) chlorophyll Content; (e) VC Content; (f) Spoilage index; (g) L* value; (h) a* value; (i) b* value. Different present significant differences between groups ($p < 0.05$). (CK: control check, T1: 1.65 g L^{-1}, T2: 2.50 g L^{-1}, T3: 3.35 g L^{-1}, T4: 4.25 g L^{-1}).

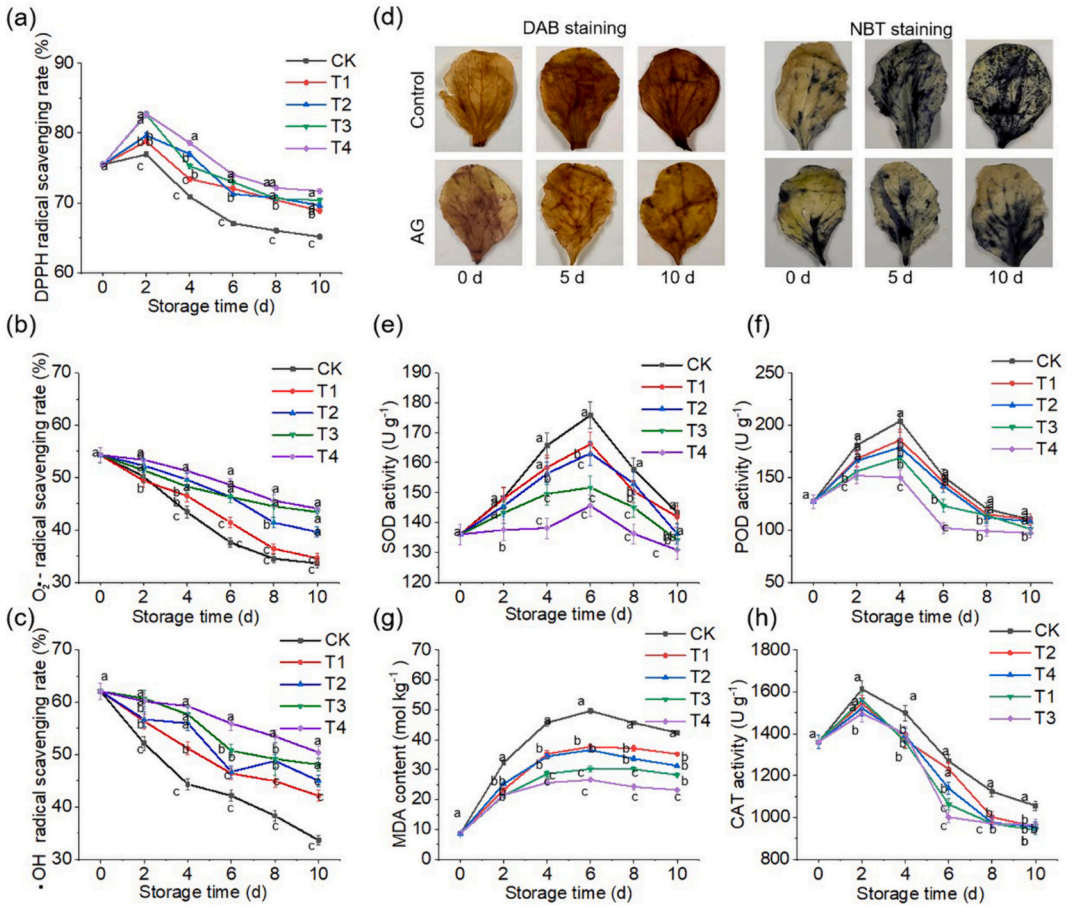


Fig. 3. The effect of AG treatment on enzyme of Pakchoi seedlings during storage. (a) DPPH radical scavenging rate; (b) $O_2\cdot^-$ radical scavenging rate; (c) ·OH radical scavenging rate; (d) DAB staining and NBT staining; (e) SOD activity; (f) POD activity; (g) MDA content; (h) CAT content. Different present significant differences between groups ($p < 0.05$). (CK: control check, T1: 1.65 g L^{-1}, T2: 2.50 g L^{-1}, T3: 3.35 g L^{-1}, T4: 4.25 g L^{-1}).

substrate within the leaves, subsequently leading to an increase in the respiration rate(Sortino et al., 2022). A lower respiration rate signifies reduced metabolic activity within the vegetables, aiding in their extended preservation duration. As seen in Fig. 2c, the respiration rate of Pakchoi seedlings was stable within 2 days after harvest, and the treated groups were lower than the CK. Thereafter, the respiration rate of all Pakchoi seedlings showed a relatively large increase. At 4 d of storage, compared with the beginning, the respiration rate of the CK increased nearly six times, while that of the T1, T2, and T4 groups increased only four times. Furthermore, as respiration involves the consumption of organic matter, this consequently leads to a reduction in TSS content, in Fig. 2b, we can see that the TSS content of T4 was 2.32%, while CK only was 1.71%, this emphasizes the AG can be able to effectively suppress the respiration intensity of Pakchoi seedlings.

Chlorophyll is the main pigment in Pakchoi seedlings, and the chlorophyll in the chloroplasts of higher plants consists of chlorophyll-a and chlorophyll-b(Sarker, Oba, Alsanie, & Gaber, 2022). During storage, the chlorophyll content (Fig. 2d), chlorophyll-a and chlorophyll-b (Fig. S1) of Pakchoi seedlings showed an overall decreasing trend. During storage, the chlorophyll content of all treatment groups was higher than that of the CK. After 6 d, the chlorophyll content of CK decreased from 1.46 g kg^{-1} to 0.75 g kg^{-1}, while the chlorophyll content of T3 and T4 was 1.11 g kg^{-1}, which was 48% higher than that of the CK. During storage, the chlorophyll-a content was higher in all treatment groups than in the CK. Chlorophyll-b content in T1 and T2 changed rapidly after 4 d, while chlorophyll content in T3 and T4 changed rapidly only after 6 d. AG can reduce the degradation of chlorophyll content and maintain the storage quality of Pakchoi seedlings.

VC is the main nutrient component of Pakchoi seedlings(Qingqing et al., 2022). Related studies found that the VC content of most vegetables decreases rapidly during storage(Zhu, Geng, & Sun, 2021). In the present experiment (Fig. 2e), the low concentration of AG solution had no effect on maintaining the VC content of Pakchoi seedlings. However, as the concentration of AG increased, it effectively inhibited the decrease in VC content in Pakchoi seedlings. At 4 d of storage, the VC content of T4 was 0.33 g kg^{-1} and that of CK was 0.27 g kg^{-1}, and T4 was 19.71% higher than CK. As shown in Fig. 2f, throughout the storage period, T1, T2, T3, and T4 decayed less than CK, and T4 had the best preservation effect of them all.

Color difference is a visual measure reflecting color variation(Perumal et al., 2022; Qian et al., 2022). The color change of Pakchoi seedlings affects its merchantability and reflects its quality to some extent (Mingying, Siyuan, Zhaoyang, & Jing, 2022). During storage, the color of Pakchoi seedlings transitioned progressively from dark green to white, accompanied by a shift in color hue. However, the application of AG delayed the increase in L* value. Within 10 d, the L* values of the treated groups were lower than those of the CK (Fig. 2g). AG maintained the color of Pakchoi seedlings better, which was especially evident in the late storage period. AG also delayed the decrease of greenness a* value in Pakchoi seedlings (Fig. 2h). During the storage period, the L* value of the treated groups was higher than that of the CK. After 4 d, the CK showed partial yellowing due to quality deterioration and had difficulty maintaining an appreciable green color, while the treated group was able to retain a green color with commercial value. It was evident that AG treatment could delay the decline in a* value. The changes in the color difference b* value of Pakchoi seedlings after harvesting are shown

in Fig. 2i. b* value represent the yellow value of the vegetable, and the b* value of Pakchoi seedlings all increased to different degrees during storage. The increase in b* value reflects the increase in the yellowing of Pakchoi seedlings. During storage, the b* value of the treated groups was all lower than those of the CK. After 4 d, the b* value of the CK surpassed that of the treated group, indicating more yellowing in the CK group. This observation aligns with Fig. 1b, where evident yellowing than the other groups can be observed in the CK. Overall, AG slowed the yellowing of Pakchoi seedlings, allowing them to retain their green color.

3.3. Effect of AG treatment on enzyme index of Pakchoi seedlings

Vegetables can draw oxygen molecules into their bodies through respiration, but they only accept one electron to convert into the $O_2\cdot^-$, from which H_2O_2, OH^-, $_1O^2$, and other radicals can be derived(Gunathilake, Ranaweera, & Rupasinghe, 2018; Meitha, Pramesti, & Suhandono, 2020; Siyuan, Zhaoyang, & Jing, 2021a; Thilavech, Marnpae, Makynen, & Adisakwattana, 2021). The use of the DPPH radical scavenging method can better reflect the strength of antioxidant activity in plants(Thilavech et al., 2021). The DPPH radical scavenging capacity exhibited a pattern of initial increase followed by a subsequent decrease during the storage of Pakchoi seedlings (Fig. 3a). Notably, it demonstrated an upward trend for the first two days, reaching its zenith at 2 d. This tendency is believed to stem from the elicitation of antioxidant activity within the fruit and vegetable in response to environmental stimuli and the potential onset of disease(Mansoor et al., 2022). However, AG significantly promoted the rise and inhibited the decline of DPPH radical scavenging capacity, which was higher in all treated groups than in the CK during the storage period, with the best DPPH scavenging capacity in T4. During storage, the $O_2\cdot^-$radical scavenging capacity (Fig. 3b) and ·OH radical scavenging capacity (Fig. 3c) generally showed a decreasing trend. After 6 d, T4 maintained a higher $O_2\cdot^-$ radical scavenging capacity throughout the storage period, and the scavenging rate was 29.68% higher than that of the CK at 10 d. However, with the increase of AG solution concentration, the scavenging capacity of $O_2\cdot^-$free radicals stopped increase. From Fig. 3c, we can see that AG has the effect of maintaining the ·OH radical scavenging capacity, and T4 maintained a high ·OH radical scavenging capacity throughout the storage period, and at 10 d, the scavenging rate was 54.32% higher than that of the CK. This indicates that AG had effect on the elevated antioxidant activity of Pakchoi seedlings.

Plant tissues often generate a range of ROS when confronted with stressful environmental conditions. Due to their high reactivity and inherent instability, the detection of ROS typically relies on their resulting end products(Siyuan, Zhaoyang, & Jing, 2021b; Sun et al., 2023). H_2O_2 represents one type of ROS. When catalyzed by CAT, H_2O_2 rapidly interacts with DAB, forming a distinctive brownish-red compound that localizes H_2O_2 within the tissues. $O_2\cdot^-$ is another type of ROS, which is an oxygen-containing free radical that reduces NBT to a water-insoluble blue metazonite, thus localizing $O_2\cdot^-$ in tissues(Kexin, Haodong, Chenwei, & Jing, 2023; D. Li et al., 2023). NBT is used for $O_2\cdot^-$ staining of living plant tissues, such as tender root tips and leaves. After staining, the areas with $O_2\cdot^-$ aggregation show a blue to dark blue color.

SOD is an enzyme that catalyzes the disproportionation of $O_2\cdot^-$ to H_2O_2 and O_2. Because the depth of color embodied in the leaves is indirectly related to the magnitude of the content of SOD and CAT, this experiment visualizes the in-situ accumulation of H_2O_2 and $O_2\cdot^-$, which can help visualize the physiological state of Pakchoi seedlings. In Fig. 3d, it can be observed that after the beginning of storage, the color of AG treated Pakchoi seedlings was much lighter than that of the CK (brownish red). Combined with the change in SOD activity, the AG-treated Pakchoi seedlings had lower SOD activity than the CK, which also verified the results of the staining reaction. Similarly, in Fig. 3d, after the beginning of storage, it can be observed that the AG-treated Pakchoi seedlings were much lighter than the CK (dark blue). The Pakchoi seedlings treated with AG showed reduced CAT activity in comparison to CK, which also verified the results of the staining reaction. The above results suggest that AG can effectively inhibit the generation of ROS.

The main function of SOD is to eliminate harmful substances produced by organisms in the process of metabolism(Liburdi, Benucci, & Esti, 2019). A sudden increase in SOD activity can serve as an indicator that the plants' physiological condition is compromised, necessitating heightened SOD activity to eliminate detrimental substances. In Fig. 3e, during storage, it is shown that the SOD activity of the five groups of Pakchoi seedlings exhibited a general trend of increasing and then decreasing. The SOD activity of CK increased faster in the first 6 d, but after 6 d, the SOD activity of CK decreased rapidly. Similarly, the SOD activity of all the five groups of Pakchoi seedlings showed an overall trend of increasing and then decreasing throughout the entire storage period. At the end of storage, the SOD activity of T4 was the lowest, only 2.32% different from that at the beginning of storage. For T3 and T4, the notable absence of a suddenly rise in SOD activity implies that the growth and physiological status of Pakchoi seedlings within the T3 and T4 remained favorable. Moreover, all SOD activity of Pakchoi seedlings treated with AG were below CK, indicating that AG could reduce the production of H_2O_2 and $O_2\cdot^-$.

POD is an important oxidoreductase enzyme in fruit and vegetables (Liburdi et al., 2019). POD can catalyze lignin monomers into phenolic radicals in the presence of H_2O_2 and polymerize to produce lignin(Chou et al., 2018). During storage in this experiment, POD activity showed an increasing trend followed by a decreasing trend (Fig. 3f). The trend of increasing and then decreasing POD activity during storage of Pakchoi seedlings is actually an antioxidant defense response mechanism of the plant(Yao et al., 2021). AG promoted the rise of POD activity in Pakchoi seedlings and inhibited the decline of activity at the later stage. At 10 d, the POD activity of the treated groups was all higher than that of the CK. Although POD can reduce the harmful effects of free radicals in living organisms, it might also result in damage to the fruit and vegetables themselves. The experimental outcomes demonstrated that AG effectively managed the swift elevation of POD activity. This action not only safeguarded the cells of Pakchoi seedlings but also decelerated the process of organ senescence, thereby extending the storage period for Pakchoi seedlings.

MDA is one of the main products of biofilm lipid peroxidation(Lin, Zhan, Shao, & Sun, 2022), and its content is used to reflect the degree of cellular biofilm lipid peroxidation. The accumulation of MDA can cause damage to the cytoplasm and organelles of fruit and vegetables(Sang et al., 2022). After harvesting, the biofilm structure of fruit and vegetables can be damaged, leading to reduced quality, increased susceptibility to pathogenic bacteria, and decreased storage resistance(D. K. Liu, Xu, Guo, & Zhang, 2020; Y. F. Zhao & Zheng, 2022). Considering the positive preservation effect of preservatives on fruit and vegetables (Rojas et al., 2022), using preservatives or preservation techniques to maintain a balance between the production and removal of ROS is a way to delay the decay of Pakchoi seedlings. As observed in Fig. 3g, there was a rapid rise in the content of MDA in Pakchoi seedlings during the pre-storage period, which could be a stress response caused by the non-adaptation of Pakchoi seedlings to the environment at the beginning of storage. The data reveals an inverse correlation between the MDA content of Pakchoi seedlings and the concentration of AG. AG has no inhibitory effects on MDA content at lower concentrations, but at higher concentrations, it prevents MDA content from decreasing.

CAT is an enzyme that catalyzes the breakdown of excess H_2O_2 in plants, thereby reducing the damage of H_2O_2 to fruit and vegetable tissues(Ali, Khan, Malik, Shaheen, & Shahid, 2018; T. T. Li et al., 2019). As seen in Fig. 3h, the CAT activity of all samples increased during the first two days of storage but then continued to decline afterward. In general, the different concentrations of AG had varying effects on whether CAT activity increased or decreased. For T3 and T4, AG increased CAT activity early in storage and delayed the decline later than other groups during storage.

3.4. Effect of AG treatment on microorganisms

Fruit and vegetables infested with microorganisms would decay faster, leading to a loss of nutritional value and posing risks to human health(Fan, Wu, & Chen, 2021). Some people use natural antimicrobial substances to reduce the rate of microbial growth and reproduction to protect these fruit and vegetables(Anacarso et al., 2011).

GA and its derivative AG are members of the triterpenoid compound family, belong to the triterpenoid compound family. Triterpenoid compounds possess the capability to disrupt bacterial cell biofilms and interfere with bacterial metabolic processes, resulting in bactericidal effects(Ren & Kinghorn, 2020). These properties make triterpenoid compounds, including GA and AG, promising candidates for various applications in the fields of medicine, food, and biotechnology. AG as a triterpenoid compound derived from natural extract GA, is not only safe and harmless, but also exhibits exceptional antibacterial properties. *E. coil*, *S.aureus*, and *B.Subtilisis* were the most common spoilage pollutant of vegetables and the main cause of spoilage of newly cut vegetables (Gutierrez, Rodriguez, Barry-Ryan, & Bourke, 2008). According to the results shown in Fig. 4a–d, with increasing AG concentrations, the inhibitory effects on *E.coil*, *S.aureus*, and *B.Subtilis* growth become more pronounced. *E.coil*, *S.aureus*, and *B.Subtilis*. Measuring bacterial oxidative stress levels allows for an assessment of AG's antibacterial efficacy. MDA levels correlate with the extent of bacterial cell membrane damage (Feng et al., 2020). As demonstrated in Fig. 4e, as AG concentration increases, the extent of bacterial damage also rises. When bacteria face environmental stress, the levels of SOD and glutathione peroxidase (GSH-Px) rise(Zhang et al., 2023). Fig. 4f and Fig. 4g illustrate AG could disrupt bacterial cell membranes to impact oxidative balance. This aligns with results in Fig. 4h, which reveal a sharp decline in intracellular ATP levels due to leakage resulting from cell membrane damage.

From Fig. 4i and Fig. 4k, it is evident that with an increase in AG concentration, the bacterial survival rate of *E.coil*, S.*aureus*, and *B.subtilis* showed a decreasing trend. This indicates that AG can effectively inhibit spoilage bacteria in vegetables. However, when the AG solution concentration exceeds 3.35 g L^{-1}, the antibacterial capacity reaches its saturation point. From Fig. 4j and Fig. 4l, the data indicates that as the concentration of AG solution increases, there is a corresponding enhancement in the inhibitory effect on the total number of colonies in Pakchoi seedlings. However, consistent with the observation in Fig. 4i, the inhibitory effect was not enhanced when the concentration of AG solution exceeded 3.35 g L^{-1}. This suggests that higher concentration of AG solution do not lead to further improvements in the inhibitory effect on bacterial colony growth. These experiments demonstrate that AG possesses antibacterial properties, effectively inhibiting microbial growth, and extending the shelf life of Pakchoi seedlings.

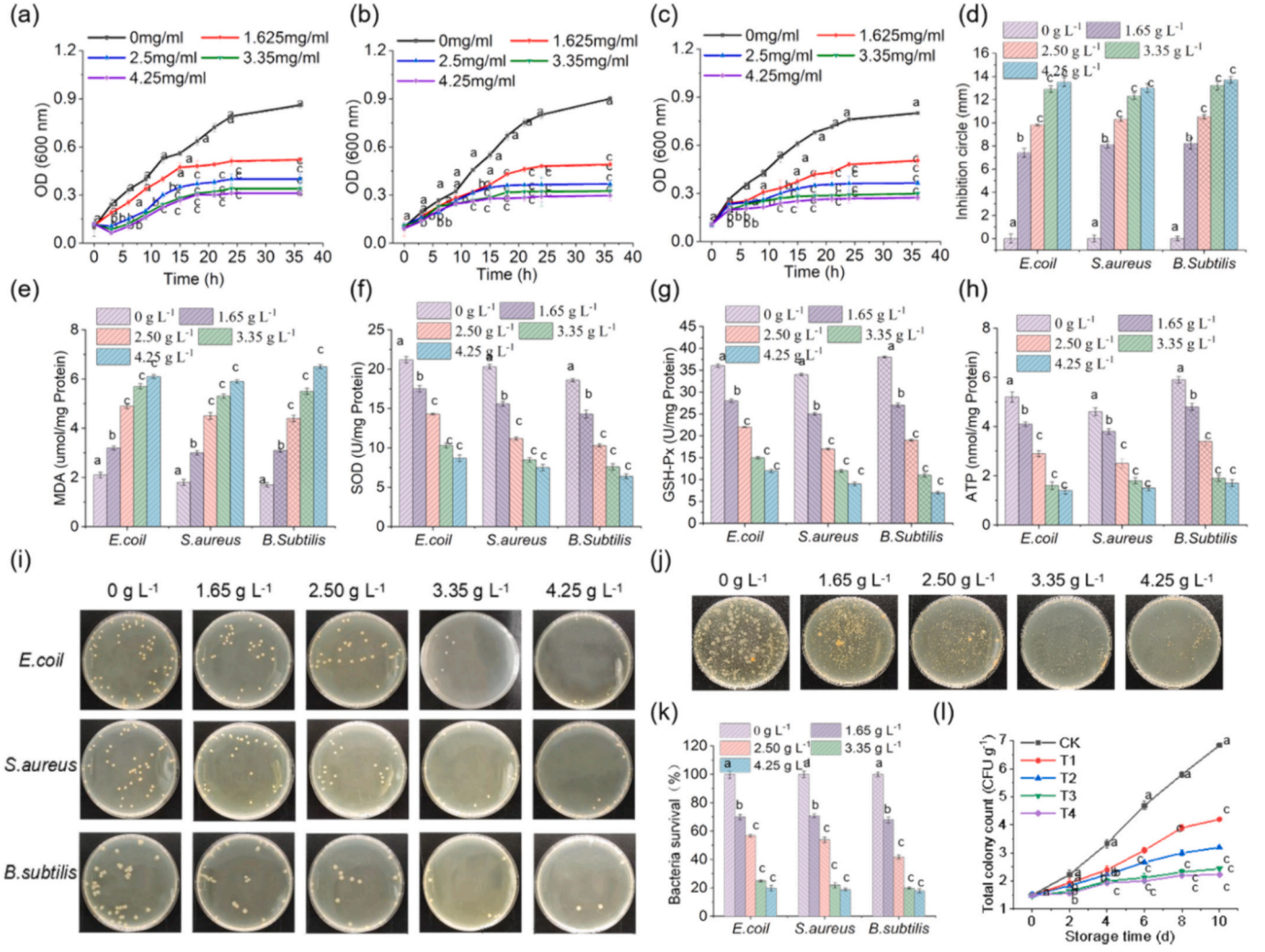


Fig. 4. Effect of different concentrations of AG solution treatment on microorganisms. (a) Growth curves of *E.coil*; (b) Growth curves of *S.aureus*; (c) Growth curves of *S.aureus*; (d) Inhibition circle; (e) MDA; (f) SOD; (g) GSH-Px; (h) ATP; (i) Effect of AG treatment on *E.coil, S.aureus*, and *B.Subtilis*; (j) Effect of AG treatment on microorganisms in surface of Pakchoi seedlings; (k) The bacterial survival rate. (l) The total colony count. Different present significant differences between groups ($p < 0.05$). (CK: control check, T1: 1.65 g L^{-1}, T2: 2.50 g L^{-1}, T3: 3.35 g L^{-1}, T4: 4.25 g L^{-1}).

3.5. Effect of AG treatment on bacterial cell biofilm

The bacterial cell biofilm formed by the aggregation of bacteria and their secretions, such as polysaccharides, lipoproteins, and fibronectin, which can withstand harmful substances and protect bacteria from damage (Shree, Singh, Sodhi, Surya, & Singh, 2023). Therefore, inhibiting the formation of biofilm is an important indicator for evaluating the antibacterial efficacy of antimicrobial agents. The strength of bacterial biofilm potential determines the degree of cell membrane breakage, and the smaller the potential, the greater the degree of cell membrane breakage. The results of bacterial biofilm potential are shown in Fig. 5a. After the addition of different concentrations of AG solution to the bacterial suspension, the fluorescence intensity of *E.coil*, *S.aureus*, and *B.Subtilis* continuously decreased, and this decrease was inversely proportional to the concentration of AG solution. This phenomenon shows that AG solution can destroy bacterial cell biofilm better. The absorbance at 570 nm of crystal violet reflects the integrity of the biofilm. The lower the absorbance, the lower the membrane integrity. As shown in Fig. 5b, compared with the blank group, the absorbance values of *E.coil*, *S.aureus*, and *B.Subtilis* gradually decreased, indicating that AG has an inhibitory effect on the formation of bacterial cell biofilm. Protein carbonylation is a type of protein oxidative damage that can reflect physiological changes in cells. The higher the content, the worse the physiological state of the cell biofilm. Fig. 5c shows that with the increase of AG solution concentration, Protein carbonylation content increased gradually. That's because AG can induce protein oxidation, which disrupts the synthesis of bacterial cell biofilms.

In summary, the antibacterial mechanism of AG is through the disruption of cell biofilm and inhibition of their synthesis.

4. Discussions

Studies clearly indicate that AG has a positive effect on the shelf life of Pakchoi seedlings. The treated group showed slower nutrient decline, stable POD and SOD activities, and effective management of MDA content, preventing damage to cell biofilm induced by excessive ROS. AG also exhibited higher $O_2\cdot^-$ and ·OH radical scavenging abilities, inhibiting CAT activity, and slowing down the yellowing rate of Pakchoi seedlings. Furthermore, AG could maintain a lower level of POD activity prevents the accumulation of free radicals. That the treated groups T1, T2, T3, and T4 all exhibited some freshness effect compared to the CK, with the T4 showing the most pronounced effectiveness among the other groups. Changes in respiration rate and water content confirmed that AG can indeed enhance freshness by obstructing the stomata of pakchoi seedlings, this is consistent with the findings of Driesenrt et al., that stomata and respiration rate of vegetables, as well as freshness, are related to the loss of nutrients(Driesen, Van den Ende, De Proft, & Saeys, 2020). Overall, compared with the CK, the AG maintained relatively better color, delayed the onset of yellowing and chlorophyll content, preserved the better appearance of Pakchoi seedlings, and delayed the senescence of Pakchoi seedlings. Moreover, the microbiological experiment demonstrated the efficacy of the AG solution as an antibacterial agent. This solution effectively inhibited microbial growth and thwarted rapid spoilage resulting from microbial invasion.

Table S2 reveals that the antioxidant properties of AG solution remained relatively stable over a period of 30 d. The DPPH radical scavenging rate decreased by only 12.93%, while the ·OH radical scavenging rate decreased by only 15.08%. Additionally, the $O_2\cdot^-$ radical scavenging rate decreased by only 12.83%. From Table S3, AG solution still has bacteriostatic stability after 60 d of placement. This antimicrobial effect played a crucial role in sustaining the commercial quality of Pakchoi seedlings over an extended duration. According to the observation results from Fig. S2, the structure of the AG in which the Pakchoi seedlings was immersed remained unchanged after it was recovered. This indicates that the AG is highly practical and functional.

AG is a natural preservative with antioxidant and antimicrobial properties, which enhance the antioxidant capacity and extend the shelf life of vegetables. While flavonoids offer similar benefits, they may cause undesirable pigmentation. Additionally, essential oil-based preservatives might impart strong odors to vegetables. Among the known free radical scavengers, AG stands out as a natural and efficient antioxidant. Moreover, the unique chemical properties of AG allow it to act as an efficient intracellular free radical scavenger. Its high lipophilicity enables it to penetrate cell membranes and neutralize harmful free radicals within cells, while its partial hydrophilicity allows it to cross into the nucleus where it can exert its antioxidant effects at a cellular level. In conclusion, AG's multifaceted benefits make it worth in food preservation. Its capacity to boost antioxidant levels without compromising sensory attributes, and its non-toxic nature(Mantovani et al., 1988) make it an appealing choice for producers and consumers seeking high-quality natural preservatives.

5. Conclusions

To explore the impact of AG on vegetable preservation, the effect of different concentrations of AG solution on the quality of fresh Pakchoi seedlings was evaluated for the first time in this study. The results indicated that micellar structure AG, as an antioxidant, could scavenge ROS and free radicals within Pakchoi seedlings. Moreover, it also disrupts the biofilm of bacterial cells and inhibits its synthesis. Specifically, Pakchoi seedlings exhibited a high capacity for free radical scavenging capacity, antioxidant activity, and bacterial inhibition after being treated with 4.25 g L^{-1} AG solution. Therefore, AG treatment could prolong the shelf life of the Pakchoi seedlings and improve their quality. This research holds significance for advancing vegetable preservation methods and optimizing the storage of fresh produce within the

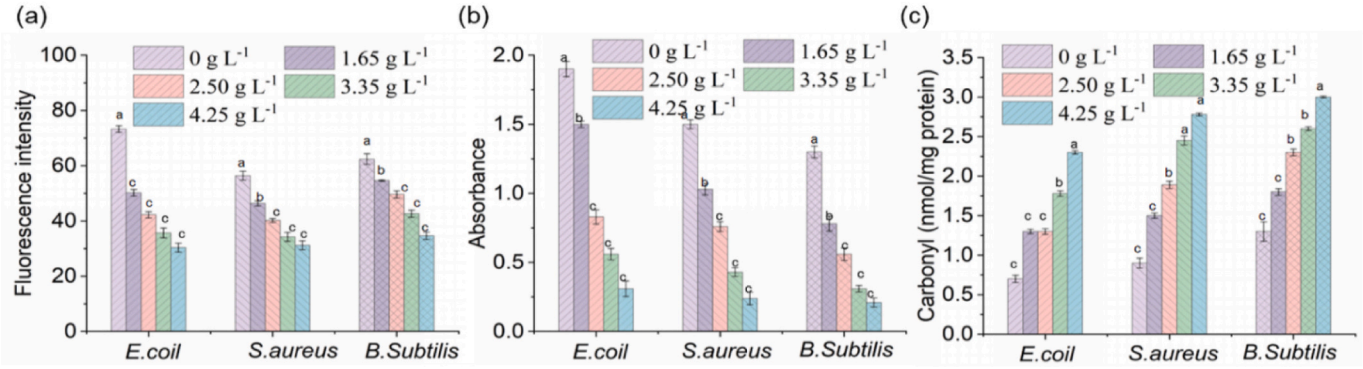


Fig. 5. The Effect of AG treatment on bacterial cell biofilm. (a) The effect of AG on cell biofilm potential of bacteria; (b) The effect of AG on bacterial biofilm formation; (c) The effect of AG on bacterial cell biofilm protein carbonyl. Different present significant differences between groups ($p < 0.05$).

agricultural industry.

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CRediT authorship contribution statement

Li Li: Software, Methodology, Conceptualization. **Zhaoyang Ding:** Writing – review & editing, Conceptualization. **Jing Xie:** Writing – original draft, Visualization, Investigation, Data curation.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101620.

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