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Effect of compound preservative treatment on the quality and physicochemical properties of fresh-cut lotus root

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Abstract: Fresh-cut lotus root is susceptible to quality problems after cutting and processing. This paper investigated the effect of 0.8% glacial acetic acid compounded with 0.5% ascorbic acid solution on fresh-cut lotus root quality and physicochemical properties. It significantly inhibited the decrease of the L^* value and the increase of a^* value, b^* value, and ΔE value, and delayed the colour change of fresh-cut lotus root. Meanwhile, the increase in weight loss rate and browning degree was postponed, and the decrease of soluble solids and hardness was inhibited. Moreover, the compound preservative treatment inhibited the activities of browning-related enzymes peroxidase (POD), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL) during storage, consequently curbing the accumulation of total phenolic content. Compared to the control group, the treatment significantly affected the increase in total colony count and altered the structure of the microbial community. In this paper, microbial sequencing results showed that the treatment significantly inhibited the growth of *Duganella* and *Janthinobacterium*. Finally, the results showed that the compound preservative delayed the quality change of fresh-cut lotus root and prolonged its shelf life by up to 10 days. This study provides a theoretical basis for further application in post-harvest fruit and vegetable preservation and processing.

Keywords: compound preservative; browning inhibition; quality maintenance; microbial community

Lotus root is one of the popular vegetables for its crisp texture and refreshing taste (Gao et al. 2017a). As an aquatic plant, it is rich in amino acids, proteins, fibre, sugars, vitamins, and other nutrients (Hu et al. 2014). Fresh-cut lotus root is gaining increasing popularity among consumers due to its convenience, cleanliness, and health benefits, thereby driving up its market demand (Wang et al. 2023). Additionally, the emergence of centralised kitchens has also provided a favourable platform for development of fresh-cut lo-

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tus root products (Zhang et al. 2022). However, once fresh-cut vegetables are peeled and sliced, exposure to air initiates a series of physiological and biochemical reactions, such as accelerated respiration, surface browning, and elevated microbial proliferation (Alegria et al. 2022). Surface browning and microbial growth are the primary causes of quality deterioration in fresh-cut lotus roots during processing (Ali et al. 2020). Enzymatic browning of lotus root is closely related to PAL (phenylalanine ammonia-lyase), PPO (polyphenol oxidase), and POD (peroxidase) (Kim et al. 2014). At present, there are some related studies on the preservation of fresh-cut lotus root. Ethanol soaking alone can inhibit *Pseudomonas* and *Pantoea* spoilage during storage of fresh-cut lotus root (Xu et al. 2023). Cysteine treatment of fresh-cut lotus roots prevented changes in the quality of fresh-cut lotus roots by inhibiting the increase in PPO activity (Wen et al. 2020). H_2S gas fumigation significantly inhibited the PPO and POD activities, and enhanced the antioxidant capacity of fresh-cut lotus root (Sun et al. 2015). In addition, a study found that treatment with 1% glutathione reduced the counts of total aerobic bacteria, yeasts, and moulds, and delayed the increase in browning degree of fresh-cut lotus root (Ali et al. 2023). 1-Methylcyclopropene-treated lotus root improved antioxidant capacity and significantly suppressed the browning response of fresh-cut lotus root (Chen et al. 2022). Most existing studies have focused on changes in the appearance and antioxidant activity of fresh-cut lotus root treated with additives, whereas few have investigated the microbial community structure during its storage.

Organic acids are widely used in the food industry for inhibiting microbial growth and enzymatic browning. Commonly used organic acids in food preservation include acetic acid, lactic acid, citric acid, and oxalic acid. Treatment with organic acids can effectively inhibit enzymatic activity, thereby reducing browning rate and prolonging the colour retention period of fresh-cut products. Acetic acid is one of the most widely used organic acids. It is often used as a preservative to inactivate food-borne pathogens (Jeong and Ha 2019). Related studies have shown that acetic acid can delay colour change in lettuce by inhibiting the activity of PAL and slowing the rate of browning (Huang et al. 2020); pre-treating potatoes with acetic acid effectively reduces nutrient loss and delays colour change (Gong et al. 2022). Ascorbic acid is a common antioxidant widely used in fruits and vegetables to break down hydrogen peroxide and reduce cellular damage through the ascorbate-glutathione cycle (Xu et al. 2020). Study (Gao et al. 2017b) investigated those low concentrations of ethanol combined with ascorbic acid significantly inhibited the increase in the microbial population of fresh-cut lotus root during cold storage and effectively delayed browning. The aim of this study is to apply 0.8% glacial acetic acid and 0.5% ascorbic acid solutions to treat fresh-cut lotus root, in order to delay quality deterioration and prolong shelf life. The quality, physicochemical properties, and microbiological characteristics of fresh-cut lotus root were systematically investigated, aiming to provide a theoretical basis for its further application in the field of post-harvest preservation and processing of fruits and vegetables.

MATERIAL AND METHODS

Main materials. The experiments used food-grade glacial acetic acid, ascorbic acid (≥ 99%), CAT (catalase) kit, POD kit, and PAL kit (Nanjing Jiancheng Technology, China), and the other analytical-grade materials were domestic brands (Chengdu Kelong Chemical, China).

Main equipment. Multiskan FC Enzyme Labeler (Thermo Fisher Scientific, Waltham, USA); DW-HL218 Ultra-low Temperature Refrigerator (Zhongke Meiling Low-Temperature Technology Hefei, China); TGL-22S High-speed Freezing Centrifuge (Sichuan Shuke Instrument, China); Testo 205 Portable pH Meter (Testo, Germany); SPX-150B constant temperature incubator (Shanghai Langan Experimental Equipment, China); SW-CJ-2FD aseptic operating table (Suzhou Antai Air Technology, China).

Sample pre-treatment. Fresh lotus roots were purchased from a local market in Chengdu, Sichuan Province. Those with uniform colour, similar size, and no visible surface damage were selected for the experiment. Fresh lotus roots were washed with tap water, peeled with a sterilised paring knife, and sliced about 4 mm. The fresh-cut lotus root were first soaked in a 0.8% glacial acetic acid solution for 5 min, then in a 0.5% ascorbic acid solution for another 5 min, and finally rinsed with distilled water. This treatment group was designated as group B, The control group A was treated with sterile water. The fresh-cut lotus root was drained, packaged in sterilised plastic-sealed bags, and then placed in a 4 °C refrigerator for storage. Sampling was conducted every two days, and the collected samples were immediately frozen in liquid nitrogen for 1 min, followed by storage in a −80 °C ultra-low temperature freezer. Three biological replicates were performed in the experiment.

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Determination of colour difference. Colour difference was measured using a portable handheld colorimeter (Liao et al. 2024). The colour parameters L^*, a^*, and b^* were measured with a colorimeter, and the ΔE value was calculated using the formula shown below.

$$\Delta E = \left[\left(L_f - L_i \right)^2 + \left(a_f - a_i \right)^2 + \left(b_f - b_i \right)^2 \right]^{1/2} \quad (1)$$

Where: L_i, a_i, and b_i – L^*, a^* and b^* values determined for fresh-cut samples on day 0 (control); L_f, a_f, and b_f – L^*, a^* and b^* values determined for the samples at each subsequent time point.

Determination of weight loss rate and browning degree. Weight loss was expressed as change in weight during storage (Xu et al. 2022a).Weight loss rate was calculated as the difference between the weight after soaking in the compound preservative treatment (W_0) and the weight before measurement (W_t), divided by W_0, and then multiplied by 100% to express it as a percentage:

$$Weight\ loss\ rate = (W_0 - W_t) / W_0 \times 100\% \quad (2)$$

The method for determining browning degree was performed according to Wang et al. (2024) with slight modifications. A total of 2 g fresh-cut lotus root tissue was mixed with 5 mL distilled water, homogenized under ice-bath conditions, and centrifuged at 10 000 rpm for 20 min. The resulting supernatant was collected and its absorbance at 410 nm was determined to quantify the browning degree.

Determination of hardness, soluble solids content. Measurement of hardness of fresh-cut lotus root using a texture tester (Martiñon et al. 2014) with the following parameters: P/6 probe, TPA (texture profile analysis) mode, 1.0 mm·s^{-1} pre-test speed, 1.0 mm·s^{-1} mid-test speed, 5.0 mm·s^{-1} post-test speed, 30% compression ratio, and a trigger force of 5 g. Each group of samples was measured in parallel ten times by using the method of averaging the results.

The soluble solids content (SSC) assay was performed using the method of Basharat (Yousuf and Srivastava 2019) with slight modifications. SSC was measured using a hand-held saccharimeter, which was standardised and zeroed with distilled water before use. A 10 g sample of fresh-cut lotus root was weighed and crushed with a mortar and pestle, the filtrate was then pressed through gauze, and the values were recorded.

Determination of PPO, POD, PAL activity, and total phenol. The activities of PPO, POD, and PAL were assayed using kits (Nanjing Jiancheng Technology Co., Ltd, China) according to the manufacturer's instructions. Total phenols were determined by referring to the method of Zhang et al. (2024) and Min et al. (2017) with slight modifications. 10 mL of methanol solution with 70% volume fraction was added to fresh-cut lotus root tissue (0.5 g), mixed well, and centrifuged (8 000 rpm·min^{-1}, 15 min). The supernatant was stored in the refrigerator at 4 °C for reserve. 0.5 mL of the extract was aspirated into a cuvette, 2.5 mL of diluted forint solution was added and was let stand at room temperature for 5 min, then 2 mL of 7.5% Na_2CO_3 solution was added; the reaction solution was placed in the dark and reacted for 2 h. The absorbance was measured at 760 nm. The sample content was quantified based on the gallic acid standard curve, and the results were expressed as total phenolic content in gallic acid equivalents per gram (mg GAE/g FW).

Determination of total colony counts. The total colony counts was determined following the method of (Wang et al. 2019) with slight modifications. An appropriate quantity of fresh-cut lotus root tissue was weighed. Then, 0.85% sterile saline was added with a 1 : 10 ratio and homogenised in a sterile sampling tape. The sample was then serially diluted to make homogenates of different dilutions. An appropriate dilution of homogenate was chosen, 1 mL of sample solution was aspirated in a sterile petri dish, and poured into the plate counting agar medium. The samples were incubated at 37 °C for 48 h and then taken out for counting. The quantity was expressed as $\log_{10}$ CFU·g^{-1}.

Analysis of bacterial community structure. The extracted DNA from fresh-cut lotus root tissue was purified and then used for PCR amplification of the 16S rRNA V3–V4 region with primers 341F (CCTAYGGGRBGCASCAG) and 806R (GGACTACNNGGGGTATCTAAT) after passing quality control. The PCR reaction system (25 μL) was configured as follows: 2 × Taq Plus Master Mix 12.5 μL, upstream and downstream primers (10 μmol·L^{-1}) 1 μL, template DNA (10–50 ng·μL^{-1}) 2 μL, and enzyme-free water to make up to 25 μL. The procedure was set as follows: hold at 98 °C for 1 min to complete the first denaturation; then hold at 98 °C for 10 s, 50 °C for 30 s, and 72 °C for 30 s for 30 cycles; then hold at 72 °C for 5 min. After that, the products were mixed in equal amounts according to the product concentration, and the products were tested, and the bands recovered after mixing. The Illumina standard library construction process was adopted: the purified PCR products were ligated with Illumina sequencing adapters, and the library

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Table 1. Sensory scoring criteria for fresh-cut lotus root

Sensory indicators	Sensory score				
	5–4	4–3	3–2	2–1	< 1
Colour	the cut surface is whitish in colour	no visible browning on the cut surface	localised browning of cut surfaces	most of the sections are browned	heavy browning of cut surfaces
Odour	weak fragrance	very weak fragrance	odourless	slightly smelly	sour and offensive odour
Texture	hard texture	slightly hard texture	a softening texture	texture softening	fully softened
Acceptance	very satisfied	satisfied	neutral	dissatisfied	very dissatisfied

was amplified by PCR. The library was subsequently purified using AMPure XP magnetic beads (Beckman Coulter, Brea, USA) to remove short fragment impurities. The concentration of the library was detected by Qubit4.0 fluorescence quantitative instrument (to ensure that the concentration was ≥ 10 ng·μL^{-1}), and the length of the inserted fragment was verified by Agilent 2100 Bioanalyzer (the target fragment range was 400–500 bp), and finally the double-ended library meeting the requirements of Illumina sequencing was constructed. The qualified library was diluted (to 2 nmol·L^{-1}), mixed in equal amounts, and double-ended sequenced using the Illumina NovaSeq 6000 PE250 platform. PhiX Control v3 library was used as a positive control to monitor the sequencing quality; The sequencing strategy was 2 × 300 bp (or 2 × 250 bp) double-ended reads, and the sequencing depth of each sample was ensured to reach 30 000–50 000 valid sequences to meet the accuracy requirements of subsequent microbial community diversity analysis (such as OTU clustering, species annotation).

Sensory evaluation. Sensory evaluation was performed according to the method of Jiang et al. (2023) with slight modification. Ten students were selected to rate this experiment's fresh-cut lotus root preservation effect in four dimensions: colour, odour, texture, and acceptance. The average value of each item was taken as the sensory index score (Table 1).

Statistical analysis. Each data result was repeated three times; mean as well as standard deviation was calculated using Excel 2016 (Microsoft, USA), variables were analysed by one-way ANOVA by SPSS (v. 27, IBM SPSS Statistics, USA). and graphs were drawn using Origin2024 software, significantly difference was referred as asterisks (**P < 0.01, *P < 0.05).

RESULTS AND DISCUSSION

Effect of compound preservative treatment on L^*, a^*, b^*, ΔE values and visual appearance. As seen from Figure 1, the L^* value decreased with the extension of storage time. The L^* value indicates the brightness of fresh-cut lotus root. The brightness of the treated group was significantly higher than that of the control group, indicating that the treatment group significantly inhibited the decrease in brightness of fresh-cut lotus roots (P < 0.01). a^* values indicate the redness, with larger values indicating a more pronounced red colour. In this experiment, the a^* values of both the treated and control groups showed an increasing trend from 1.43 to 4.44 and 1.82 for the control and treated groups, respectively, indicating the treatment group significantly delayed the reddening of fresh-cut lotus root during refrigeration (P < 0.01). The b^* values of both groups showed an increasing trend during this period, and it can be seen that the change in the control group was higher than that in the treated group. ΔE value indicates the total colour difference, the main parameter to reflect the browning. Higher values of colour difference indicate greater browning. The colour difference values between the control and treated groups were highly significant (P < 0.01). It was confirmed that the applied treatment not only delayed the elevation of colour difference values in fresh-cut lotus roots but also effectively inhibited browning. In conclusion, immersion of fresh-cut lotus root in a combined solution of 0.8% glacial acetic acid and 0.5% ascorbic acid significantly inhibited discoloration, thereby effectively delaying browning.

Effect of compound preservative treatment on weight loss, browning degree. Weight change is one of the critical indicators of the quality of fruits and vegetables during storage. As shown in Figure 2A, the weight loss rates of the control and treatment groups showed a gradually increasing trend during storage. There was no significant difference between the control and treated groups during the whole period, while the weight loss rate of the control group was higher than that of the treated group on day 2 and 4 (P < 0.05). Moreover, the weight loss rates of the control and treated groups reached 1.93% and 1.74%

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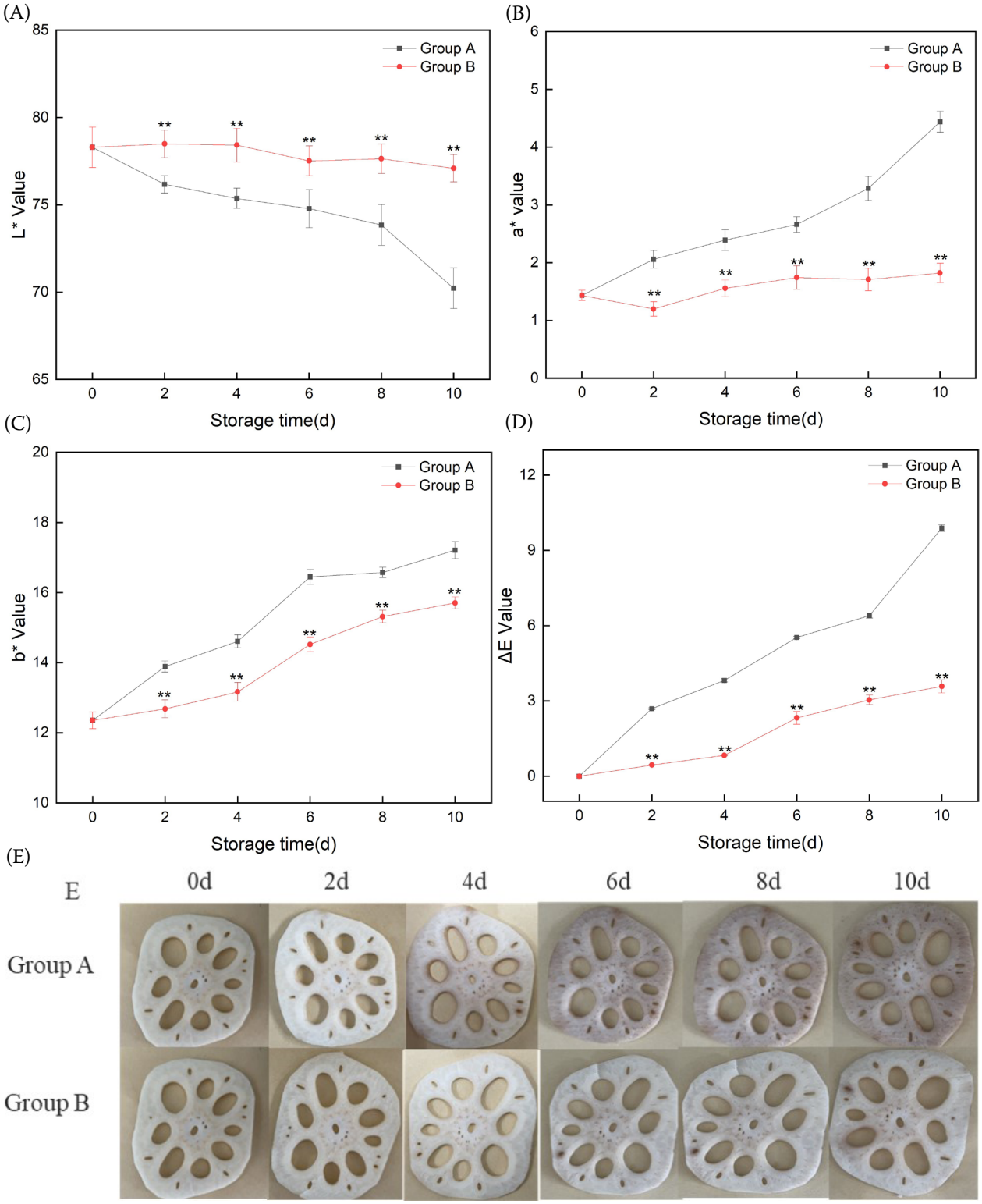

Figure 1. Changes in L^* value (A), a^* value (B), b^* (C), ΔE (D), and visual appearance (E) of different treatments under 4 °C
*significant difference at $P < 0.05$ between two groups; ** highly significant difference at $P < 0.01$; group A – control group; group B – treatment group

at day 10, respectively ($P < 0.05$). This indicates that the weight loss rate of the treated group underwent a lesser degree of change than that of the control group.

Browning degree is an essential indicator of quality change in fruits during storage time. On day 6, the control group exhibited a significantly greater browning

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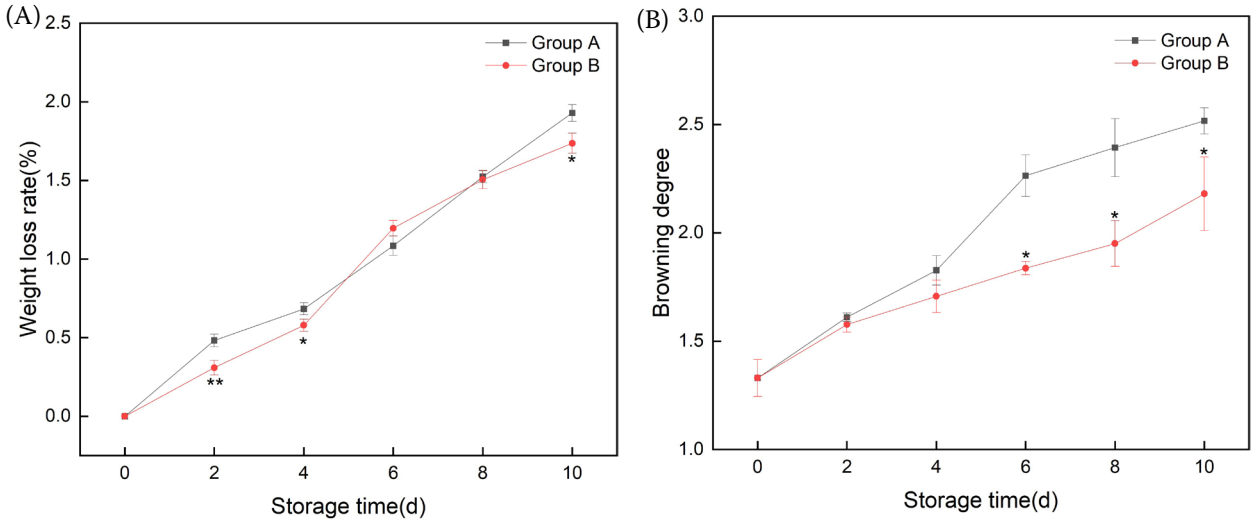

Figure 2. Changes in weight loss (A) and browning degree (B) of different treatments under refrigeration at 4 °C
*significant difference at $P < 0.05$ between two groups; ** highly significant difference at $P < 0.01$; group A – control group; group B – treatment group

degree compared to the treated group, with the former being approximately 43% higher than the latter ($P < 0.05$). Furthermore, there was a significant difference between the two groups ($P < 0.05$) on the 8th and 10th day. Ascorbic acid (vitamin C) can reduce quinones produced in enzymatic browning to phenols through its own oxidation, block the key path of further polymerisation of quinones to form brown pigments (such as melanin), and cut off the browning process from the middle of the reaction chain. Moreover, ascorbic acid has antioxidant properties, and its use in fruits and vegetables can effectively reduce the degree of oxidation and thus delay the onset of browning (Xiong et al. 2025). In this study, results demonstrated that the combined treatment effectively delayed the increase in browning of fresh-cut lotus root during refrigeration, with changes consistent with those observed in colour values.

Effect of compound preservative treatment on hardness and soluble solids (SSC). The hardness of the control and treated groups showed a decreasing trend throughout the cold storage period (Figure 3A). There was no significant difference between the hardness changes of the control and treated groups ($P > 0.05$). The hardness of the control and treated groups decreased from 4 495.71 to 3 182.45 g·f and 3 418.93 g·f, respectively, while the hardness of the treated fresh-cut lotus root decreased slowly.

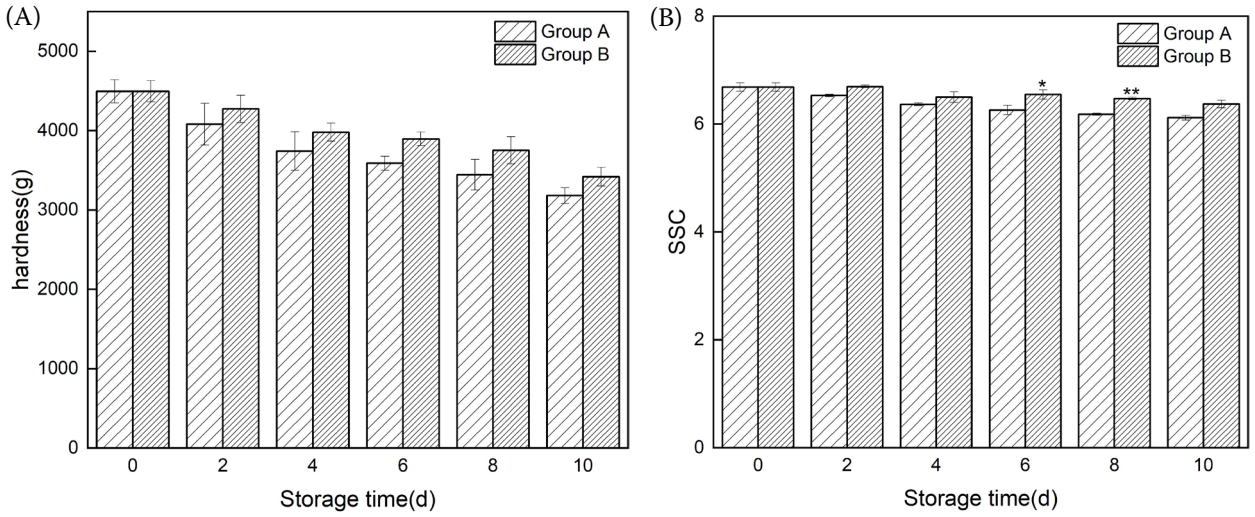

Figure 3. Changes in hardness (A) and soluble solids content (SSC) (B) of different treatments under refrigeration at 4 °C
*significant difference at $P < 0.05$ between two groups; ** highly significant difference at $P < 0.01$; group A – control group; group B – treatment group

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As seen from Figure 3B, the slight decrease in soluble solids content in the control and treatment groups may be due to reduced sugars due to respiratory depletion (Yu and Shi 2021). There was a significant difference ($P < 0.05$) between the SSC value of the control and treated group on days 8 and 10. At the end of the period, the SSC decreased by 0.57 and 0.31 in the control and treated groups, respectively ($P < 0.05$). This means that the soluble solids of fresh-cut lotus roots in the treatment group changed less, indicating that the integrity of cell structure was well preserved, and the physiological metabolism and material loss process were effectively inhibited, which is of great significance to maintain the quality and nutritional value of products. Moreover, fresh-cut lotus roots still undergo physiological metabolism such as respiration after harvest, and some soluble solids may be consumed in the process (for example, sugars are decomposed as respiratory substrates). Soluble solids in the treatment group changed little, indicating that the respiratory intensity of fresh-cut lotus root was inhibited, the overall physiological activity was at a low level, and the consumption rate of nutrients was delayed.

Effect on PPO, POD, PAL activity, and total phenols. PPO is one of the primary enzymes responsible for enzymatic browning: it oxidises polyphenols in fruits and vegetables to produce coloured quinones, which then undergo polymerisation to form melanin pigments (Massolo et al. 2011). The difference between the control and treated groups was significant by six days of treatment ($P < 0.01$). The control group showed a faster rise in PPO activity at day 6, reaching a maximum value of 6.33 U·g^{-1} PPO activity of the treatment group reached a maximum value of 5.33 U·g^{-1}

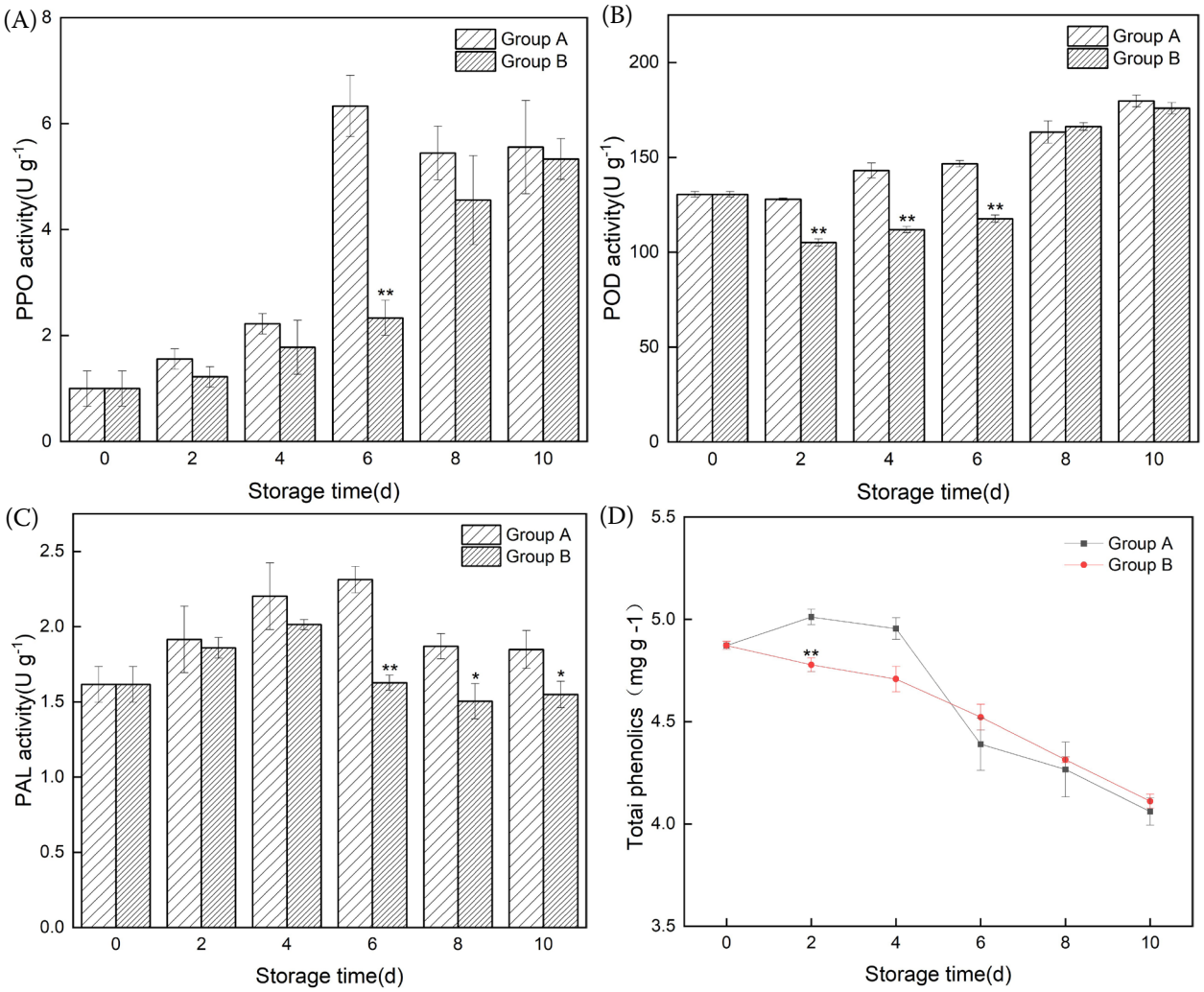

Figure 4. Changes in PPO activity (A), POD (B), PAL (C) and total phenols (D) of different treatments under refrigeration at 4°C

*significant difference at $P < 0.05$ between two groups; ** highly significant difference at $P < 0.01$; group A – control group; group B – treatment group; PPO – polyphenol oxidase; POD – peroxidase; PAL – phenylalanine ammonia-lyase

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at day 10, indicating that the treatment group could significantly delay the appearance of the peak PPO activity (Figure 4A). Due to the cleavage effect, the membrane-bound polyphenol oxidase is released from the cell membrane, becoming soluble polyphenol oxidase (S-PPO). This soluble form then interacts with browning substrates, leading to an increase in overall PPO activity (Orenes-Piñero et al. 2006). The combined use of glacial acetic acid and ascorbic acid may inhibit PPO activity by inhibiting the S-PPO from contacting the substrate. Acetic acid inactivates PPO via two key actions: it lowers the pH to disrupt the Cu^{2+}-histidine coordination in the enzyme's active site, and weakly chelates Cu^{2+} (an essential cofactor) to irreversibly reduce catalytic activity. Ascorbic acid acts as a competitive inhibitor, competing with o-diphenols for PPO's active site, and reduces Cu^{2+} to catalytically inactive Cu^{+}. Critically, acetic acid's acidic environment stabilises ascorbic acid, preventing its auto-oxidation and prolonging its inhibitory effect.

POD is one of the critical enzymes involved in enzymatic browning. The enzyme activity was lower in the first six days in the treated group. On the sixth day, the POD activity was 117.67 $U \cdot g^{-1}$, about 28.93 $U \cdot g^{-1}$ lower than the control group. The POD activity of the control group was significantly higher than that of the treated group on the 2nd, 4th, and 6th day ($P < 0.01$) (Figure 4B). Peroxidase (POD) catalyses the oxidative polymerisation of phenolic compounds with hydrogen peroxide as a co-substrate; this polymerisation reaction generates brown pigments, thereby causing browning in fruits and vegetables (Jiang et al. 2014). The antioxidant property of ascorbic acid may slow down the action of the POD by preventing the oxidation of phenolic compounds and thus reducing the accumulation of brown matter. Acetic acid moderately inhibits POD by acidifying the environment, which alters the conformation of its heme active site. Ascorbic acid acts as a sacrificial substrate for POD (preferentially oxidised over phenolics) and directly scavenges H_2O_2 (POD's obligatory oxidant), effectively depleting the substrate required for POD-catalysed phenolic oxidation.

PAL is a key rate-limiting enzyme in the phenylpropanoid metabolic pathway in plants. Although PAL is not directly involved in enzymatic browning, it indirectly affects the browning process of fresh-cut lotus root by regulating the synthesis of phenolic compounds (the core substrate of browning), and is an important enzyme connecting 'secondary metabolism' and 'quality deterioration'. The PAL activities of the two groups tended to increase and decrease with storage time. The PAL activity of the control group reached the highest value of 2.31 $U \cdot g^{-1}$ on the 6th day. The treatment group reached the peak value of 2.01 $U \cdot g^{-1}$ on the 4th day ($P > 0.05$) (Figure 4C). PAL activity positively correlated with the formation of phenolics, which in turn provided substrates for the enzymatic browning reaction of fruits and vegetables. Throughout the entire storage period, the PAL activity of the treated group was lower than that of the control group. This indicates that the composite treatment effectively inhibited PAL activity, thereby delaying the browning of fresh-cut lotus root. Acetic acid directly inhibits PAL activity by lowering the pH below its alkaline optimum (pH 7.0–8.5), causing conformational changes that impair substrate binding, and downregulates PAL gene expression to reduce de novo phenolic synthesis. Ascorbic acid indirectly suppresses wound-induced PAL activation by scavenging reactive oxygen species (ROS), which act as signalling molecules for PAL upregulation. Together, they reduce the upstream supply of phenolic substrates.

Free polyphenols are polyphenolic compounds in plant tissues that are not bound to cell wall components and can be directly dissolved in water or polar solvents. They are the core substrates of enzymatic browning of fresh-cut fruits and vegetables, and their content and existence directly affect the browning process and product quality. The total phenol content in the control group tended to increase and decrease. The later stage decreased because more substrates were required for enzymatic browning, and the phenolics were consumed more rapidly. The total phenolic content of the treated group decreased gradually, indicating that phenolic substances were not produced in large quantities during the early storage period. From day 6 to the end, the rate of total phenol decrease was also significantly lower than that of the control group, indicating that the enzymatic browning in the treated group consumed less substrate and had a low rate of browning (Figure 4D). The accumulation of total phenols is closely associated with browning (Dong et al. 2016). For the composite treatment group, the reduced increase in total phenols helped to mitigate the occurrence of browning reactions.

Ascorbic acid is a strong reducing agent. It rapidly donates electrons to the highly reactive *o*-quinones, reducing them back to colourless *o*-diphenols. Reaction: *o*-quinone + 2 ascorbic acid → *o*-diphenol + 2 dehydroascorbic acid (DHA). This reaction is thermodynamically favourable and effectively short-cir-

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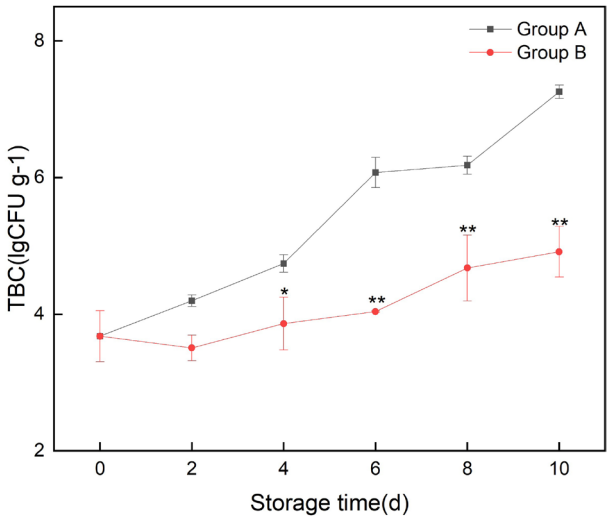

Figure 5. Changes in the total number of colonies of different treatments under refrigeration at 4 °C
*significant difference at $P < 0.05$ between two groups; ** highly significant difference at $P < 0.01$; group A – control group; group B – treatment group

cuits the browning pathway by recycling the substrates and preventing the formation of coloured polymers. Quinones are highly unstable and readily undergo non-enzymatic polymerisation or react with amino acids/proteins to form melanoidins (brown pigments). By quickly reducing quinones, ascorbic acid prevents these secondary reactions, thus inhibiting the formation of insoluble brown pigments.

Effect on total colony counts. The growth and reproduction of microorganisms are the critical factors affecting the preservation of fruits and vegetables. According to 'Green Food-Lotus Root and Its Products of China' (NY/T 1044-2020), the total colony count of fresh-cut lotus root should not exceed 5 $\log_{10}$ CFU·g^{-1}. In the control group, the colonies reached 4.73 on the 4th day and rose to 6.04 on the 6th day, which significantly exceeded the maximum limit of colony count. The total number of colonies in the treatment group reached 4.91 at the end from 3.68 at the beginning, and the total number of colonies did not exceed the national standard limit during the whole refrigeration period (Figure 5). The difference between the control and treated groups was significant during the entire storage period ($P < 0.01$).

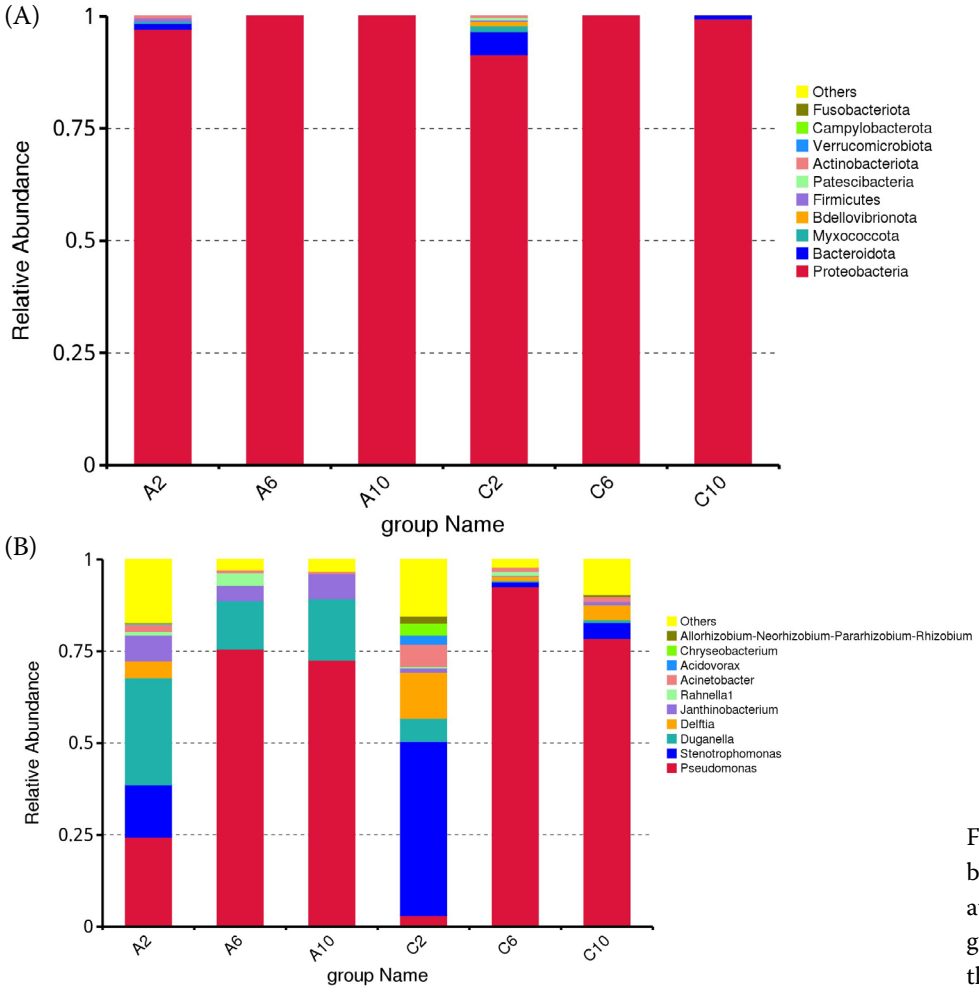

Figure 6. Changes in microbial community structure at the phylum level (A) and genus level (B) during the cold storage period

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These results demonstrated that the composite treatment of 0.8% glacial acetic acid and 0.5% ascorbic acid significantly suppressed the increase in the total colony count of fresh-cut lotus roots. Acetic acid has significant bactericidal properties, which can destroy the microbial cell structure and change the microbial growth environment.

Effect on bacterial community structure during storage of fresh-cut lotus root. The structure of the bacterial community at the phylum level for the different treatments during the storage period is shown in Figure 6A. Proteobacteria took absolute leadership in both control and treatment groups. Proteobacteria accounted for 97.14% of the initial A2, while Bacteroidota, Myxococcota, Firmicutes, and Actinobacteria phylum only 1.33, 0.38, 0.76, and 0.38%, respectively. The treated group had 91.43% of Ascomycetes, 5.14, 1.33, 0.95, 0.38, 0.57 and 0.19% of Bacteroidota, Myxococcota, Bdellovibrionota, Firmicutes, Patescibacteria, and Actinobacteriota phylum in the initial stage, respectively. Proteobacteria is the largest bacterial phylum and encompasses a large number of Gram-negative species Proteobacteria accounts for a high proportion on the surface of fruits and vegetables. Its core is that its physiological characteristics are highly compatible with the surface microenvironment of fruits and vegetables, which can accurately utilise the 'survival resources' provided by fruits and vegetables and adapt to external fluctuations. The surface of fruits and vegetables will continue to secrete or exude a variety of nutrients, and the metabolic capacity of Proteobacteria bacteria can efficiently use these substances. Moreover, some Proteobacteria (such as *Pseudomonas*) can also use pectin, cellulose and other polysaccharides released from damaged parts of fruits and vegetables, which are more likely to multiply in slightly damaged parts of fruits and vegetables. This may explain why the proportion of Proteobacteria is high in the fresh-cut lotus root.

Figure 6B illustrates the bar charts of the relative abundance of Top10 species at the genus level on days 2, 6, and 10 for the control and treated groups, respectively. There were differences in the dominant species in the initial stage between the two groups of samples, with the control group having the highest relative abundance of *Duganella*, *Pseudomonas*, and *Stenotrophomonas*. At the mid-storage period, the dominant strain in both control and treated groups was *Pseudomonas*. The relative abundance of *Pseudomonas* in the treated group reached 92.57%. As Gram-negative bacteria commonly found in fresh vegetables, *Pseudomonas* is highly viable, can grow in various environments, and is the main genera responsible for fresh-cut vegetables (Xu et al. 2022b). In the middle and late stages of storage, the percentage of *Duganella* and *Janthinobacterium* was significantly reduced in the treated group compared to the control. *Duganella* is an aerobic, motile Gram-negative bacterium, often found on plant surfaces, in soil, etc. *Janthinobacterium* can exist on the surface of fresh-cut vegetables, the core of which is that its physiological characteristics are highly com-

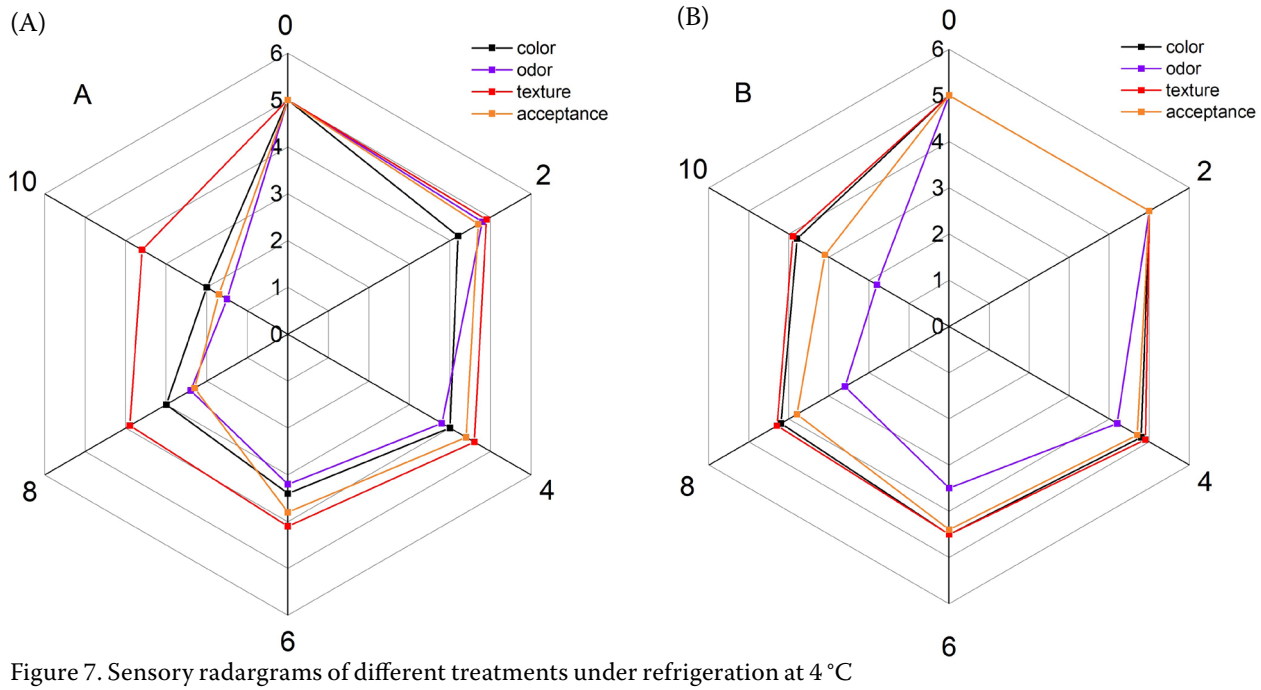

Figure 7. Sensory radargrams of different treatments under refrigeration at 4 °C

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patible with the 'damage microenvironment' of fresh-cut vegetables, which can not only utilize the nutrients brought by fresh-cut treatment, but also adapt to the environmental conditions after treatment. The reduced abundances of *Duganella* and *Janthinobacterium* indicate a **decreased spoilage risk** and **enhanced microbial community stability** in fresh-cut lotus root. *Duganella* reduction inhibits microbe-mediated enzymatic browning and tissue softening, while *Janthinobacterium* decline eliminates the primary causative agent of slimy spoilage and reduces potential toxin production. Collectively, the depletion of these two spoilage-associated genera facilitates the succession of a **non-spoilage-dominant microbial community**, strengthening the competitive exclusion against exogenous pathogens and improving the overall microbial safety and quality stability during storage.

Sensory evaluation. Sensory evaluation is one of the critical indicators of the quality of fruits and vegetables. Figures 7A and 7B represented the sensory radargrams of the control and treated groups, respectively. All indicator scores decreased with storage time. The more significant changes in the odour indicators of the treated group could be attributed to soaking in glacial acetic acid, lowering the fresh root's pH value to a weakly acidic state, and resulting in an acidic odour at the later stages of storage, but this odour was acceptable throughout the cold storage period. The control group's colour, odour, texture, and acceptance decreased from 5 on day 0 to 2, 1.5, 3.6, and 1.7, respectively. The treated group decreased from 5 on day 0 to 3.8, 1.8, 3.9, and 3.1, respectively. The results indicated that the treated group showed better overall sensory scores than the control group.

CONCLUSION

In this study, the reductions in the weight loss rate, soluble solids, hardness, and the increase in the degree of browning became slower after the treatment. Due to the cleavage effect, membrane-bound PPO is released from the cell membrane and converted into S-PPO. The combined application of glacial acetic acid and ascorbic acid can inhibit PPO activity by blocking the contact between S-PPO and its substrate, thereby improving the appearance quality and extending the shelf life of fresh-cut lotus roots. The composite treatment effectively inhibited PAL activity, reduced the synthesis of phenolic compounds, and thereby further delayed the browning of fresh-cut lotus roots. Moreover, at the genus level, the percentage of *Duganella* and *Janthinobacterium* in the treatment group was significantly reduced in the middle and late stages, indicating that the treatment group significantly inhibited the growth of *Duganella* and *Janthinobacterium*. These results indicated that 0.8% glacial acetic acid compounded with 0.5% ascorbic acid solution could delay the quality change of fresh-cut lotus root. This study only observed the changes of physical and chemical indices after treatment, and did not deeply analyse the molecular mechanism of composite treatment (such as how glacial acetic acid inhibits the expression of PAL enzyme gene, how ascorbic acid eliminates the browning intermediates quinones), and the specific mechanism needs further study. Future studies could systematically screen for browning-related differentially expressed genes (DEGs) in fresh-cut lotus root using RNA-seq technology, and accurately identify the corresponding differential metabolites via LC-MS/MS. Through the integration of multi-omics data, a gene-metabolite interaction regulatory network can be constructed, thereby clarifying the molecular regulatory pathways and core mechanisms underlying browning in fresh-cut lotus root.

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