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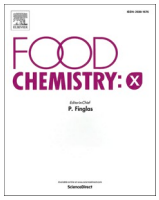

Slightly acidic electrolyzed water treatment enhances the quality attributes and the storability of postharvest litchis through regulating the metabolism of reactive oxygen species

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ABSTRACT

Effects of slightly acidic electrolyzed water (SAEW) on the storability, quality attributes, and reactive oxygen species (ROS) metabolism of litchis were investigated. Results showed that SAEW-treated litchis presented better quality attributes and storability than control litchis. On storage day 5, the commercially acceptable fruit rate of control litchis was 42%, while SAEW-treated litchis displayed 59% higher rate of commercially acceptable fruit, 21% lower pericarp browning index, and 13% lower weight loss percentage than control litchis. Additionally, compared to control litchis, SAEW-treated litchis demonstrated higher activities of SOD, CAT and APX, higher levels of GSH, AsA, DPPH radical scavenging ability, and reducing power, but lower $O_2^{\cdot-}$ generation rate, lower levels of H_2O_2 and MDA. These findings indicated that SAEW treatment could elevate antioxidant capacity and ROS scavenging ability, reduce ROS production and accumulation, and lower membrane lipid peroxidation, thereby retaining the quality attributes and storability of litchis.

1. Introduction

Litchi (*Litchi chinensis* Sonn.), a fruit native to China, has gained immense popularity because of its delightful taste and rich nutrients (He et al., 2020). However, litchi fruit ripens at summer with high temperature and humidity, the harvested litchi fruit has active physiological metabolism, and is highly susceptible to spoilage within 2–3 d, leading to severe losses of fruit quality and market value (Deshi et al., 2021; Yun et al., 2020). Litchi fruit can be preserved for a longer period under refrigeration at 5 °C and 90% relative humidity (RH), and can be sold for long-distance through cold-chain transportation (Ali et al., 2019; Deshi et al., 2021). However, due to the high cost of cool room and cold-chain system equipment, it is not available in many litchi production areas. Currently, sulfur dioxide fumigation serves as the major commercial method for prolonging the storage life of litchis in postharvest handling (Bai, Yang, Shen, Shao, & Zeng, 2022; Wu et al., 2017). However, this method is banned in many countries due to the fact that the fruit absorbs a significant amount of sulfur, ranging from 30% to 60% during fumigation, exceeding the permitted threshold (10 mg kg^{-1}) (Deshi et al., 2022; Siddiqui, Deshi, Homa, Aftab, & Aftabet, 2021). Hence, it is pressing needed to develop an innovative, environmentally friendly, and safe litchi preservation technology.

Slightly acidic electrolyzed water (SAEW) is defined as electrolyzed oxidizing water with an available chlorine concentration (ACC) ranging

Abbreviations: ACC, available chlorine concentration; AEW, acidic electrolyzed water; ANOVA, analysis of variance; APX, ascorbate peroxidase; AsA, ascorbic acid; ATP, adenosine triphosphate; C3G, cyanidin-3-glucoside; CAT, catalase; DHA, dehydroascorbate; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EDTA, ethylenediaminetetraacetic acid; GSH, glutathione; H_2O_2, hydrogen peroxide; 1-MCP, 1-methylcyclopropene; MDA, malondialdehyde; NBT, nitrogen blue tetrazole; $O_2^{\cdot-}$, superoxide anion radical; ORP, oxidation-reduction potential; PBS, phosphate buffer solution; RH, relative humidity; ROS, reactive oxygen species; SAEW, slightly acidic electrolyzed water; SOD, superoxide dismutase; TA, titratable acidity; TSS, total soluble solids.

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from 10 mg kg^{-1} to 80 mg kg^{-1}, a pH of 5.0–6.5, and an oxidation-reduction potential (ORP) of ≥900 mV (Chen et al., 2020; Cheng et al., 2023; Sun et al., 2022). SAEW not only provides a physical cooling for fresh produce, but also has strong antibacterial property, safety, and easy preparation (Gao, Yang, Bi, & He, 2023; Sun et al., 2022; Zhang et al., 2023). SAEW is a legal food additive in the United States, Korea, and Japan, and is considered a safe postharvest approach for fresh produce (Gao et al., 2023; Xuan et al., 2017). It was demonstrated that SAEW could maintain the storability and superior quality of various fruits, such as cherry tomatoes, strawberries, apples, and mandarins (Zhang, Cao, & Jiang, 2021). Zhang, Liu, et al. (2023) reported that higher levels of nutritional values and quality were found in SAEW treated-carambola. SAEW could delay color change, prevent the increase of cell membrane permeability, and reduce the fruit's respiration rate. It suggested that SAEW treatment might be a valuable approach to enhance the storability of harvested carambola (Zhang, Liu, et al., 2023). Cheng et al. (2023) also found that SAEW treatment combined with a low voltage electrostatic field remarkably decreased the moisture loss, inhibited the hardness decline, maintained the total phenol content, and boosted the storability of fresh-cut pineapples.

Previous studies have demonstrated that the storability and quality of fresh produce could be influenced by the accumulation of reactive oxygen species (ROS) and the capability of scavenging ROS (Chen, Hung, Chen, Lin, & Lin, 2019; Huang, Wang, Bi, & Xiang, 2022; Lin, Chen et al., 2020; Lin, Lin et al., 2020). Under the normal storage conditions, the generation rate of $O_2^{\cdot-}$ and the malondialdehyde (MDA) level of fruit increase with the extension of storage time, accompanied by the reduced levels of ascorbic acid (AsA), glutathione (GSH), reducing power, and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging ability, leading to excessive accumulation of ROS and the accelerated decline of quality (Lin, Lin, Lin, Fan, & Lin, 2021; Zhang, Shan, et al., 2023). Conversely, Huang et al. (2022) found that the malic acid combined with lycopene could markedly retard the increase of MDA level, and elevate the level of reducing power and the capability to scavenge DPPH radical, thereby keeping better cell structure and function of litchis, which helped maintain the quality of postharvest litchi fruit. Thus, we hypothesized that ROS metabolism played a crucial role in enhancing the quality properties and storability of postharvest litchi fruit. However, it remained unclear regarding the impacts of SAEW on the quality properties, storability, and ROS metabolism of litchi fruit during postharvest storage. Therefore, this work aimed to examine the impacts of SAEW on the nutritive properties, storability, MDA and H_2O_2 levels, generation rate of $O_2^{\cdot-}$, non-enzymatic antioxidant level, and antioxidant enzyme activities in harvested litchis. This research may provide new theoretical basis for effectively and safely maintaining the quality of postharvest litchi fruit.

2. Materials and methods

2.1. The instruments and main reagents

A slightly acidic hypochlorite water generator BD-600 L (Shanghai Fu-Qiang-Wang Sanitary Products Co., Ltd. Shanghai, China) was utilized to generate SAEW. An ORP redox potentiometer (JIA-BEI Water Treatment Co., Ltd., Guangdong, China) was employed to determine the ORP value of SAEW. The ACC value of SAEW was detected by colorimetric method using a high-concentration effective salt meter (Kasahara Chemical Instruments Corp, Saitama, Japan).

All chemicals used in this experiment were analytical grade. Sodium dihydrogen phosphate, disodium hydrogen phosphate, ethylenediaminetetraacetic acid (EDTA), α-naphthylamine, thiobarbituric acid, and nitroblue tetrazole were purchased from Shanghai McLean Biochemical Technology Co., Ltd. (Shanghai, China). Trichloroacetic acid, titanium tetrachloride, sulfuric acid, and H_2O_2 were purchased from Shanghai Linen Technology Development Co., Ltd. (Shanghai, China). Hydroxylamine hydrochloride, *p*-aminobenzenesulfonic acid, and AsA were purchased from Aladdin Reagent Shanghai Co., Ltd. (Shanghai, China). Acetone, hydrochloric acid, and ammonia were purchased from China National Pharmaceutical Group Shanghai Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Litchi fruit and its treatment

Litchi (*Litchi chinensis* Sonn. cv. Zhuang yuan hong) fruit at commercial maturity was collected from an orchard (Nan'an, Fujian, China). Pericarp color was used to evaluate the fruit maturity. >80% of litchi pericarp with bright red was considered to reach the commercial maturity, and the color parameters L^*, a^*, b^*, and C in litchi pericarp were 35.4, 29.6, 17.5, and 33.4, respectively. The postharvest litchi fruit was delivered to our laboratory in Fuzhou within 3 h. After being trimmed off the fruit stems, the fruit with consistent weight and size and without visual defects, cracks, and disease symptoms were chosen. Then the chosen litchi fruit was washed with tap water, and was dried in the air. Subsequently, the air-dried litchi fruit was used for the following treatment.

A preliminary test was performed to screen the optimal SAEW for litchi fruit. Litchi fruit was immersed in SAEW (ORP = 938 mV; pH = 6.0) with various ACC values of 0, 20, 40, 60, and 80 mg L^{-1} for 10 min. Subsequently, the treated fruit was dried in the air and kept at 25 ± 1 °C and 90% RH. After 6 d of storage, the browning index of litchi pericarp was determined. The values of browning index were ranked: 3.83 (40 mg L^{-1} ACC) < 4.08 (20 mg L^{-1} ACC) < 4.46 (60 mg L^{-1} ACC) < 4.47 (80 mg L^{-1} ACC) < 5.14 (control). Based on the rank, SAEW containing 40 mg L^{-1} of ACC was selected for this experiment.

The above 180 air-dried fruits were sampled to assess the initial indicators of litchi fruit on the harvest day (0 d). The remaining 2520 litchi fruits were randomly divided into two groups (1260 fruits/group). One group was fully submerged in SAEW (ACC = 40 mg L^{-1}) for 10 min. The other group was set as control, soaking in sterile distilled water for 10 min. After being dried in the air under an ambient condition, the samples were sealed in polyethylene film bags (thickness = 0.015 mm) (30 fruits/bag, 42 bags/group). All samples were stored for 7 d under the conditions of 25 ± 1 °C and 90% RH. Within the storage period, 6 bags (180 fruits) from each group were daily sampled for assessing the following indicators.

2.3. Determinations of commercially acceptable fruit rate, weight loss percentage, and pericarp browning index

Referring to the methods of Jiang et al. (2018a) and Liu, Lin, Lin, Lin, and Fan (2021), a total of 30 individual litchi fruits were selected to assess the commercially acceptable fruit rate. Commercially acceptable fruit rate (%) = (the number of commodity fruit / the number of total fruit) × 100%.

Following the protocols described by Javed et al. (2023), Jiang et al. (2018a) and Liu et al. (2021), one bag (30 fruits) of litchis was used to evaluate the percentage of weight loss. The data was presented as %.

Thirty litchis were applied to appraise the pericarp browning index following the approaches described by Deshi et al. (2022), Huang et al. (2022) and Jiang et al. (2018a).

2.4. Determinations of color characteristics, pericarp chlorophyll and anthocyanin contents

Following the approaches described by Jiang et al. (2018a) and Liu et al. (2021), a chroma meter (Konica Minolta Sensing, Inc., Osaka, Japan) was used to determine the chromaticity values (L^*, a^*, b^* and C) of litchi pericarp. Two spots on opposite sides of the equatorial perimeter of ten individual litchi fruits were measured. Referring to the method of Beyaz, Ozturk, and Turker (2010), ΔE* represented the overall color change of litchi fruit during storage. The calculation formula was as follows: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

Following the protocols of Lin et al. (2019) and Liu et al. (2021), pericarp tissues (one gram) from 10 litchi fruits were used to quantify the content of chlorophyll and anthocyanin in litchi pericarp, and its level was respectively indicated as mg kg^{-1} and mg cyanidin-3-glucoside (C3G) kg^{-1} based on the fresh weight of the pericarp.

2.5. Determinations of the contents of pulp total soluble solids (TSS), total soluble sugar, sucrose, reducing sugar, titratable acidity (TA), and vitamin C

Following the protocols described by Chen et al. (2020), Jiang et al. (2018a) and Liu et al. (2021), pulp tissues (ten grams) from 10 litchi fruits were sampled to determine the contents of TSS, total soluble sugar, sucrose, reducing sugar, TA, and vitamin C.

2.6. Determinations of pericarp $O_2^{\cdot-}$ production rate, H_2O_2 content, and MDA content

The rate of $O_2^{\cdot-}$ production was determined according to the methods of Lin et al. (2014), Lin, Lin, et al. (2020) and Tang et al. (2020). Pericarp tissues (1 g) from 10 litchi fruits were ground with 8 mL of 50 mmol L^{-1} phosphate buffer solution (PBS, pH 7.8, containing 1 mmol L^{-1} EDTA), and centrifuged for 20 min at 15000 ×*g* and 4 °C. One milliliter of supernatant, one milliliter of 50 mmol L^{-1} PBS (pH 7.8), and one milliliter of 1 mmol L^{-1} hydroxylamine hydrochloride were mixed and reacted at 25 °C for 1 h. Then one milliliter of 17 mmol L^{-1} *p*-aminobenzenesulfonic acid and one milliliter of 7 mmol L^{-1} *α*-naphthylamine were added, and the color development was continued for 20 min. Then the absorbance value of the reaction solution was detected at 530 nm, and the unit of $O_2^{\cdot-}$ production rate was mmol kg^{-1} min^{-1}.

The determination of H_2O_2 content followed the method of Lin, Chen, et al. (2020). Pericarp tissues (1 g) from 10 litchi fruits were ground with 8 mL of acetone, and centrifuged for 15 min at 15000 ×*g* and 4 °C. One milliliter of the supernatant was taken and mixed with 0.1 mL of 10% titanium tetrachloride (dissolved in concentrated hydrochloric acid) and 0.2 mL of concentrated ammonia water for 5 min. It was then centrifuged again for 15 min and the resulting precipitate was washed three times with acetone. The precipitate was dissolved in 3 mL of 1 mmol L^{-1} sulfuric acid, and the absorbance value of the obtained solution was measured at 412 nm. The unit of H_2O_2 content was mol kg^{-1}.

The MDA content was determined with reference to the methods of Lin et al. (2014), Lin, Lin, et al. (2020) and Huang et al. (2022). Pericarp tissues (1 g) from 10 litchi fruits were ground with 8 mL of 10% trichloroacetic acid, and centrifuged for 15 min at 15000 ×*g* and 4 °C. Three milliliters of the supernatant were mixed with 3 mL of 0.67% thiobarbituric acid and reacted in boiling water for 20 min, then the reaction solution was cooled, and its absorbance value at 450 nm, 532 nm, and 600 nm was measured. The unit of MDA content was μmol kg^{-1}.

2.7. Determinations of pericarp antioxidant enzyme activities

The activities of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) were quantify according to the approaches described by Lin et al. (2014), Lin, Lin, et al. (2020), Xie et al. (2022) and Li et al. (2024). In short, pericarp tissues (1 g) from 10 litchi fruits were ground with 8 mL of 50 mmol L^{-1} PBS (pH 7.0), and centrifuged for 20 min at 15000 ×*g* and 4 °C. The supernatants were collected to assay the activities of SOD, CAT and APX. The SOD activity was quantified by monitoring the ability to inhibit the photoreduction of nitrogen blue tetrazole (NBT), the absorbance value of the reaction solution was detected at 560 nm. The CAT activity was quantified by monitoring the decomposition of H_2O_2, the absorbance value of the reaction solution was detected at 240 nm. The quantitative method for APX activity was to mix 0.2 mL of enzyme solution with 4 mL of 50 mmol L^{-1} PBS (pH 7.7, containing AsA) and 0.5 mL of 2 mmol L^{-1} H_2O_2, then react at 30 °C for 20 min, add 2 mL of 20% trichloroacetic acid to terminate the reaction, and finally measure the absorbance value of the solution at 290 nm.

Protein content of above enzyme solution was detected following the approach described by Bradford (1976), and the unit of enzyme activity was expressed as U mg^{-1} protein.

2.8. Determinations of pericarp GSH and AsA contents

Following the previously reported approaches (Jiang et al., 2018b; Lin et al., 2014; Lin, Lin, et al., 2020), pericarp tissues (1 g) from 10 litchi fruits were used to quantify GSH and AsA levels, and its unit was expressed in g kg^{-1} based on the fresh weight of litchi pericarp.

2.9. Determinations of pericarp DPPH radical scavenging ability and reducing power

Following the methodologies of Lin, Lin, et al. (2020) and Huang et al. (2022), pericarp tissues (1 g) from 10 litchi fruits were used to assay DPPH radical scavenging ability and reducing power, and its unit was expressed in % and g kg^{-1}, respectively.

2.10. Statistical analysis

Measurement of the above indicators was conducted in triplicates. SPSS 22.0 software was executed to statistically analyze the collected data. All the data in the figures were expressed as the mean ± standard error ($n = 3$). The symbol * and ** in the figures respectively indicated significant differences ($p < 0.05$) and highly significant differences ($p < 0.01$) between the SAEW-treated litchis and the control litchis on the same day of storage, according to one-way ANOVA and independent sample *t*-test. Additionally, the asterisk * or ** in the correlation coefficient *r* value represented the correlation at the level of $p < 0.05$ or $p < 0.01$, separately.

3. Results

3.1. Pericarp browning, commercially acceptable fruit rate, and weight loss percentage

Fresh litchi pericarp showed the best appearance quality on the day of harvest, with a bright red color and no browning (Fig. 1A, B). However, along with the extension of storage time, the pericarp browning of litchis gradually appeared, transitioning from bright red to yellow-brown, resulting in a decline in the commercial value. The symptoms and index of pericarp browning in the control group slowly increased during 0–4 d, and rapidly increased within the following three days of storage. Whereas, for SAEW-treated group, the pericarp browning was effectively suppressed, and the pericarp appearance quality was maintained during the whole stages of storage, as evidenced by lower pericarp browning index than the control group. Moreover, SAEW-treated fruit displayed a notably lower index of pericarp browning than the control fruit on day 4, 5, 6, and 7, with reductions of 26%, 21%, 27%, and 10%, respectively (Fig. 1A, B).

After one day of storage, the commercially acceptable fruit rate in the control group significantly decreased, whereas, for SAEW-treated litchis, it began to decrease after two days of storage (Fig. 1C). During storage days 2–7, SAEW-treated fruit showed a higher rate of commercially acceptable fruit than the control fruit (Fig. 1C).

The percentage of weight loss in the control samples increased slowly during the initial one day of storage, and then increased quickly after one day of storage (Fig. 1D). However, during storage days 2–7, SAEW-treated litchis showed a lower percentage of weight loss. For example, on days 6 and 7, SAEW-treated fruit presented 8% and 17% lower weight loss than the control fruit, respectively.

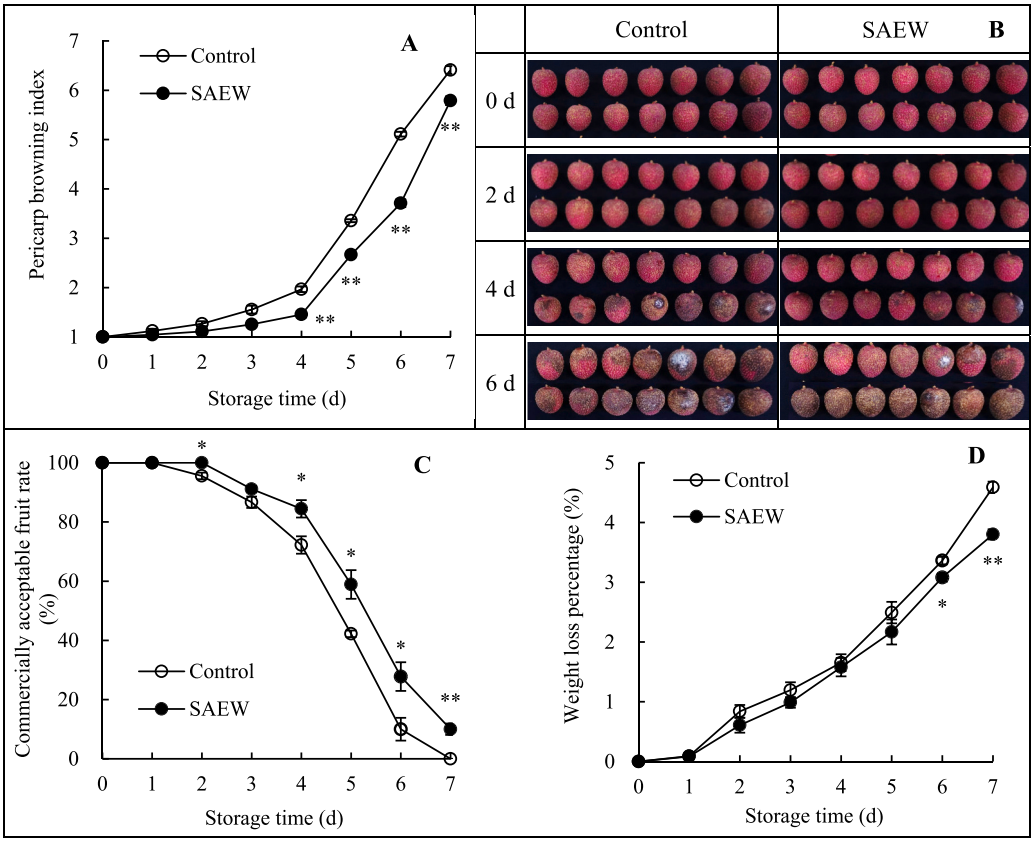

Fig. 1. Effects of SAEW treatment on the pericarp browning index (A), pericarp appearance quality (B), commercially acceptable fruit rate (C) and weight loss percentage (D) in litchi fruit during storage. The data in the figures were expressed as the mean ± standard error ($n = 3$). The symbol * and ** in the figures respectively indicated significant differences ($p < 0.05$) and highly significant differences ($p < 0.01$) between the SAEW-treated litchis and the control litchis on the same day of storage, according to one-way ANOVA and independent sample *t*-test. ○, Control litchis; ●, SAEW-treated litchis. ANOVA, analysis of variance; SAEW, slightly acidic electrolyzed water.

3.2. Color characteristics, pericarp chlorophyll and anthocyanin contents

As depicted in Fig. 2A-D, throughout the entire storage period, the gradual decreases in the chromaticity values (L^*, a^*, b^*, and C) were observed in both groups. However, during storage, the higher levels of those chromaticity values were displayed in SAEW-treated fruit as compared to the control fruit. For example, on day 6, higher levels of 6% for chromaticity L^*, 36% for chromaticity a^*, 25% for chromaticity b^*, and 23% for chromaticity C were shown in SAEW-treated fruit than those in the control fruit. Additionally, SAEW-treated fruit displayed a lower ΔE^* value compared to the control group within storage days 1–7 (Fig. 2G), indicating a milder color change during storage.

The contents of chlorophyll and anthocyanin in litchi pericarp showed a tendency to drop as the storage period prolonged (Fig. 2E, F). However, the decreases in the contents of pericarp chlorophyll and anthocyanin were effectively inhibited by the SAEW treatment. For example, on day 7, the contents of chlorophyll and anthocyanin in the pericarp of SAEW-treated litchis were 34% and 37% higher than those of the control fruit, respectively.

3.3. Pulp TSS, total soluble sugar, sucrose, reducing sugar, TA, and vitamin C contents

As illustrated in Fig. 3A, the TSS level in pulp of the control fruit climbed during 0–2 d, reached its maximum value on day 2, and dropped appreciably within 2–7 d. Compared with the control group, during storage, SAEW-treated litchis maintained a higher content of pulp TSS, with significant differences on day 1, day 3, and days 5–7. For example, on days 6 and 7, SAEW-treated fruit presented 12% and 10% higher TSS level than the control fruit, respectively.

Fig. 3 B and 3C showed that, the levels of total soluble sugar and sucrose in pulp of the control fruit reduced with the extension of storage time. However, the SAEW treatment effectively inhibited the decreases in the levels of pulp total soluble sugar and sucrose, thereby sustaining higher levels of these compounds. On day 6, SAEW-treated fruit showed 8% higher total soluble sugar and 52% higher sucrose content than the control fruit.

The reducing sugar content in pulp of litchi fruit manifested an increment trend during 0–3 d and presented a reducing trend afterward (3–7 d) (Fig. 3D). The peaks of the reducing sugar content in both groups were found on day 3, reaching 71.10 g kg^{-1} and 72.86 g kg^{-1} in SAEW-treated litchis and the control litchis, respectively. Lower levels of reducing sugar were observed in SAEW-treated fruit than the control fruit within 1–7 d. For example, on day 7, SAEW-treated fruit showed 4% lower level of reducing sugar than the control fruit.

As shown in Fig. 3E, the TA level in pulp of the control fruit dropped rapidly during storage 0–4 d and ascended afterward (4–7 d). It is worth noting that the TA level in pulp of SAEW-treated litchis displayed a similar variation pattern. The lowest level of TA in the control group was found on day 4, whereas the SAEW treatment showed the lowest level of TA on day 5. On day 7, SAEW-treated group displayed 25% lower level of TA than the control group (Fig. 3E).

As depicted in Fig. 3F, the vitamin C content of the control litchi pulp declined gradually within 0–3, and declined rapidly afterward (3–7 d). The vitamin C level in pulp of SAEW-treated litchis followed a similar changing pattern. Compared to the control group, SAEW-treated litchis showed higher level of vitamin C during storage, with significant differences between day 4 and day 7. On day 7, SAEW-treated fruit

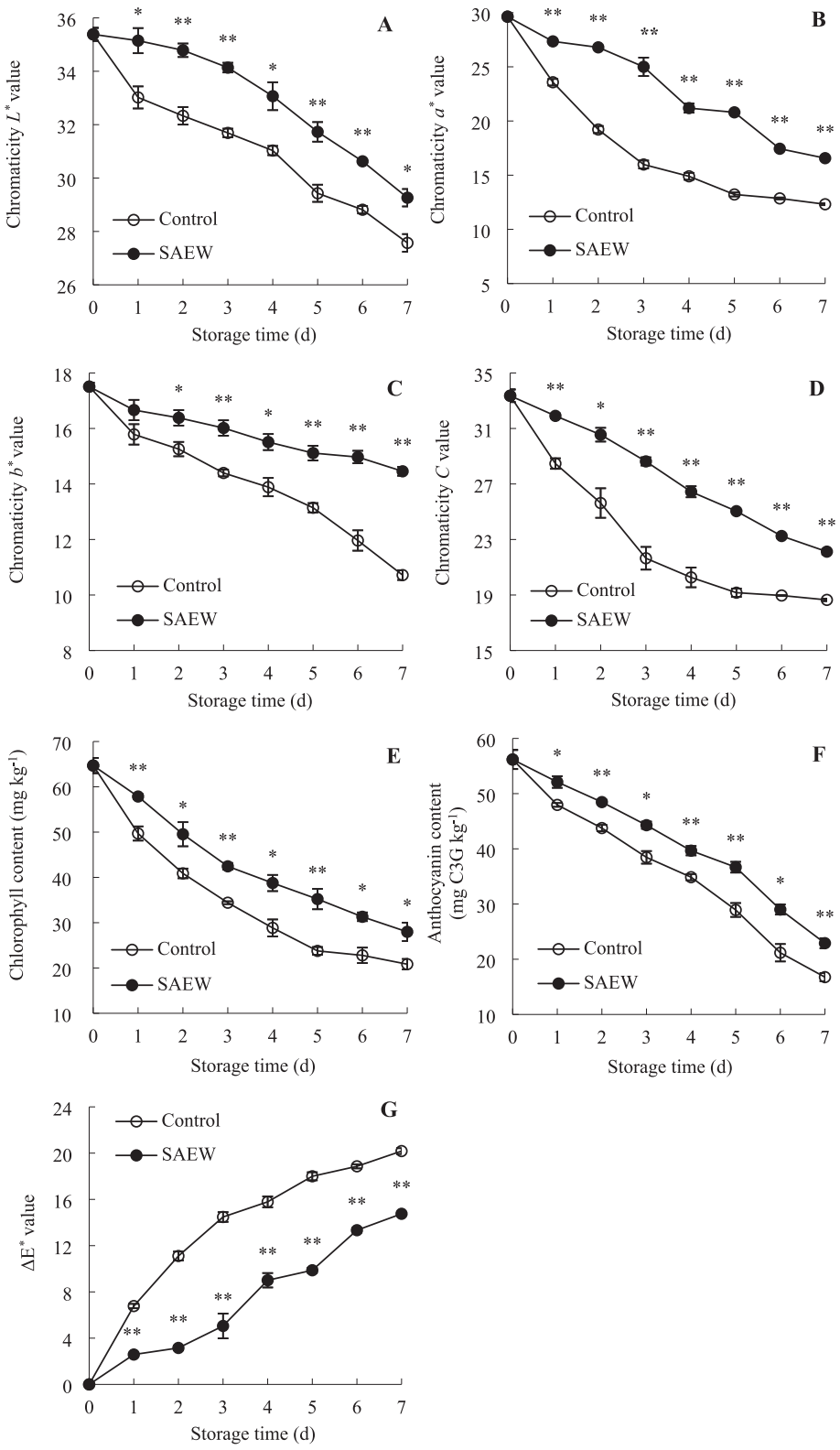

Fig. 2. Effects of SAEW treatment on the chromaticity L^* value (A), chromaticity a^* value (B), chromaticity b^* value (C), chromaticity C value (D), chlorophyll content (E), anthocyanin content (F) and ΔE^* value (G) in pericarp of litchi fruit during storage. The data in the figures were expressed as the mean ± standard error (n = 3). The symbol * and ** in the figures respectively indicated significant differences ($p < 0.05$) and highly significant differences ($p < 0.01$) between the SAEW-treated litchis and the control litchis on the same day of storage, according to one-way ANOVA and independent sample t-test. ○, Control litchis; ●, SAEW-treated litchis. ANOVA, analysis of variance; SAEW, slightly acidic electrolyzed water.

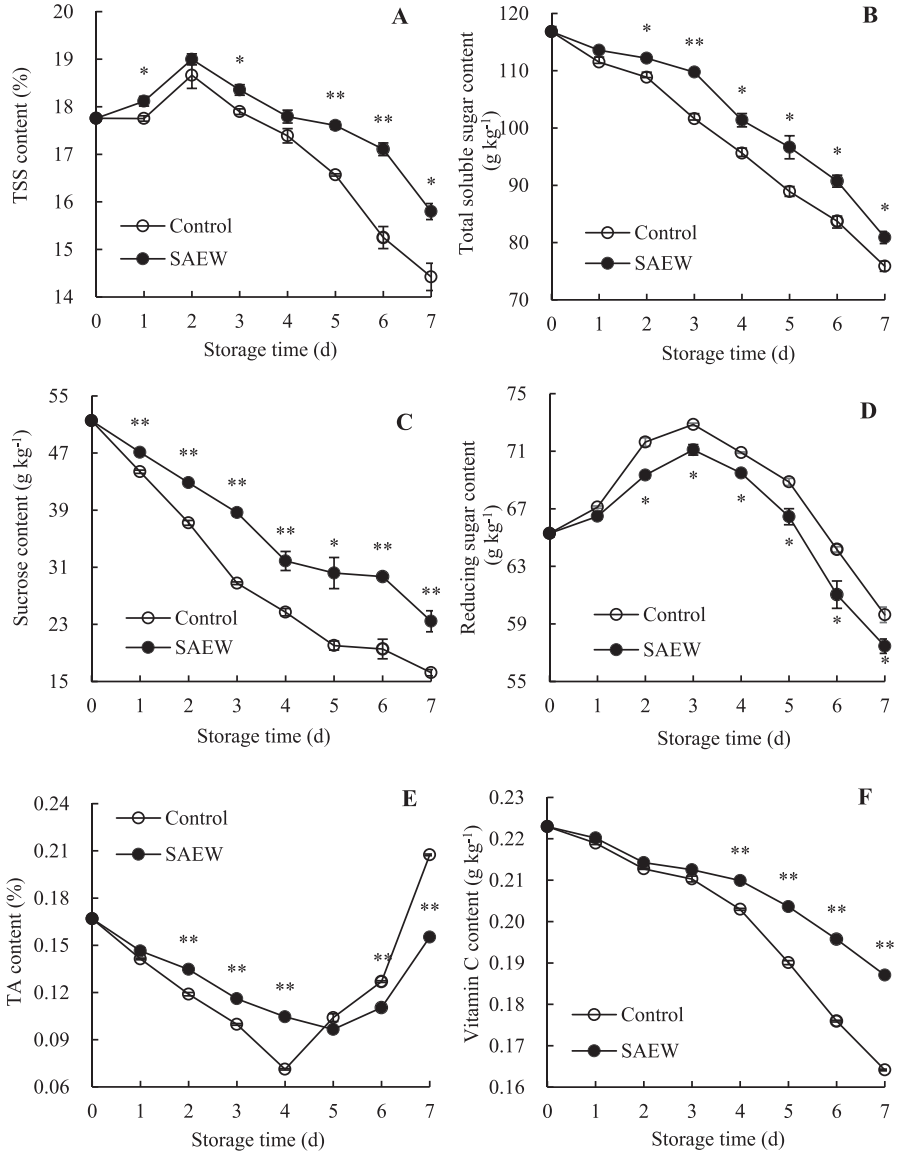

Fig. 3. Effects of SAEW treatment on the contents of TSS (A), TA (B), vitamin C (C), total soluble sugar (D), sucrose sugar (E), and reducing sugar (F) in pulp of litchi fruit during storage. The data in the figures were expressed as the mean ± standard error (n = 3). The symbol * and ** in the figures respectively indicated significant differences ($p < 0.05$) and highly significant differences ($p < 0.01$) between the SAEW-treated litchis and the control litchis on the same day of storage, according to one-way ANOVA and independent sample *t*-test. ○, Control litchis; ●, SAEW-treated litchis. ANOVA, analysis of variance; SAEW, slightly acidic electrolyzed water; TSS, total soluble solid; TA, titratable acidity.

exhibited 14% higher level of vitamin C than the control fruit.

3.4. Pericarp $O_2^{\cdot-}$ production rate, H_2O_2 level, and MDA content

The $O_2^{\cdot-}$ generation rate in the pericarp of two groups displayed an upward tendency from day 0 to day 5, peaked on day 5, and then reduced dramatically during 5–7 d (Fig. 4A). In addition, compared to the control fruit, lower level of $O_2^{\cdot-}$ generation rate was shown in SAEW-treated fruit during storage, with significant differences on days 2–7 except day 3. For example, on day 7, SAEW-treated fruit presented 30% lower level of $O_2^{\cdot-}$ generation rate than the control fruit.

Fig. 4B and C demonstrated that the H_2O_2 and MDA contents of the litchi pericarp on harvest day were 2.33 mol kg^{-1} and 1.56 μmol kg^{-1}, respectively. Compared to the control fruit, SAEW treatment inhibited the increases of H_2O_2 and MDA contents, and maintained lower levels of H_2O_2 and MDA during storage, with significant differences within days 5–7. On day 7, SAEW-treated fruit displayed 11% lower level of H_2O_2 and 32% lower content of MDA than those in the control fruit.

3.5. Pericarp antioxidant enzymes activities

SOD activity in pericarp of the control litchi fruit showed slight changes over 0–5 d and ascended rapidly in the next two days (Fig. 5A). During storage, SAEW-treated fruit exhibited higher SOD activity, with 86%, 100% and 85% higher level of SOD on day 4, day 5, and day 6, respectively, as compared to the control fruit.

CAT activity in pericarp of the control litchi fruit slightly increased during 0–1 d, decreased during 1–2 d, showed no significant changes during 2–3 d, rapidly increased during 4–6 d, and then decreased during 6–7 d (Fig. 5B). During the entire stage of storage, SAEW-treated fruit showed higher activity of CAT compared to the control fruit. For example, on day 5, CAT activity (121.86 U mg^{-1} protein) in SAEW-treated fruit exhibited a significantly higher level than that in the control fruit (44.99 U mg^{-1} protein).

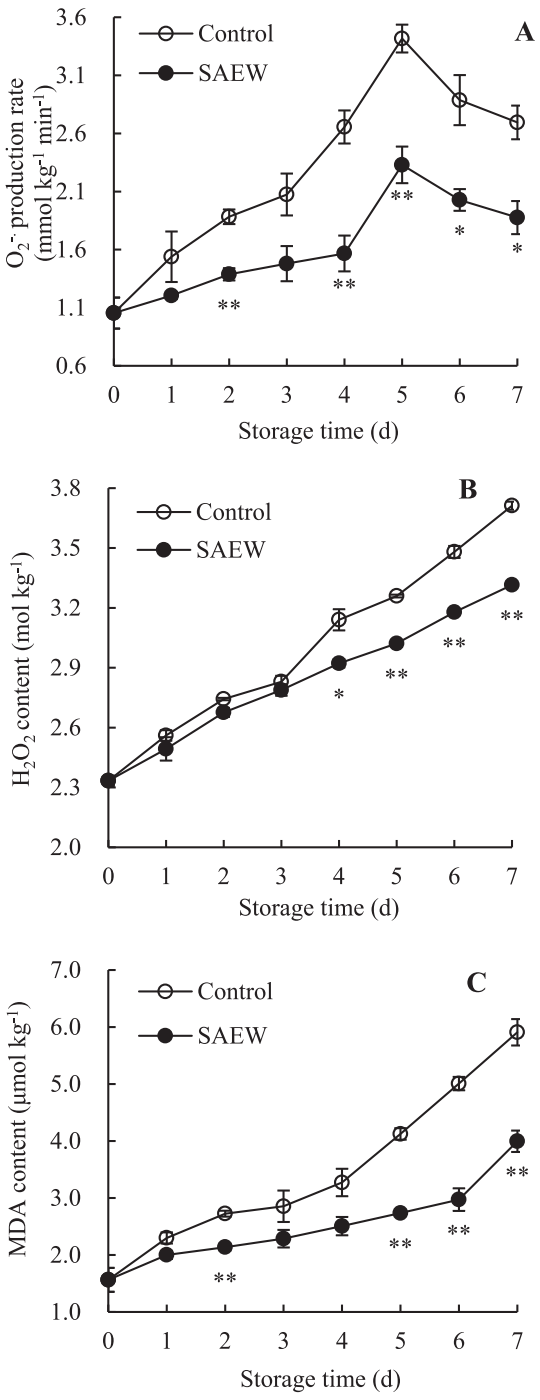

Fig. 4. Effects of SAEW treatment on the $O_2^{\cdot-}$ production rate (A), H_2O_2 content (B), and MDA content (C) in pericarp of litchi fruit during storage. The data in the figures were expressed as the mean ± standard error (n = 3). The symbol * and ** in the figures respectively indicated significant differences ($p < 0.05$) and highly significant differences ($p < 0.01$) between the SAEW-treated litchis and the control litchis on the same day of storage, according to one-way ANOVA and independent sample *t*-test. ○, Control litchis; ●, SAEW-treated litchis. ANOVA, analysis of variance; SAEW, slightly acidic electrolyzed water; $O_2^{\cdot-}$, superoxide anion radical; H_2O_2, hydrogen peroxide; MDA, malondialdehyde.

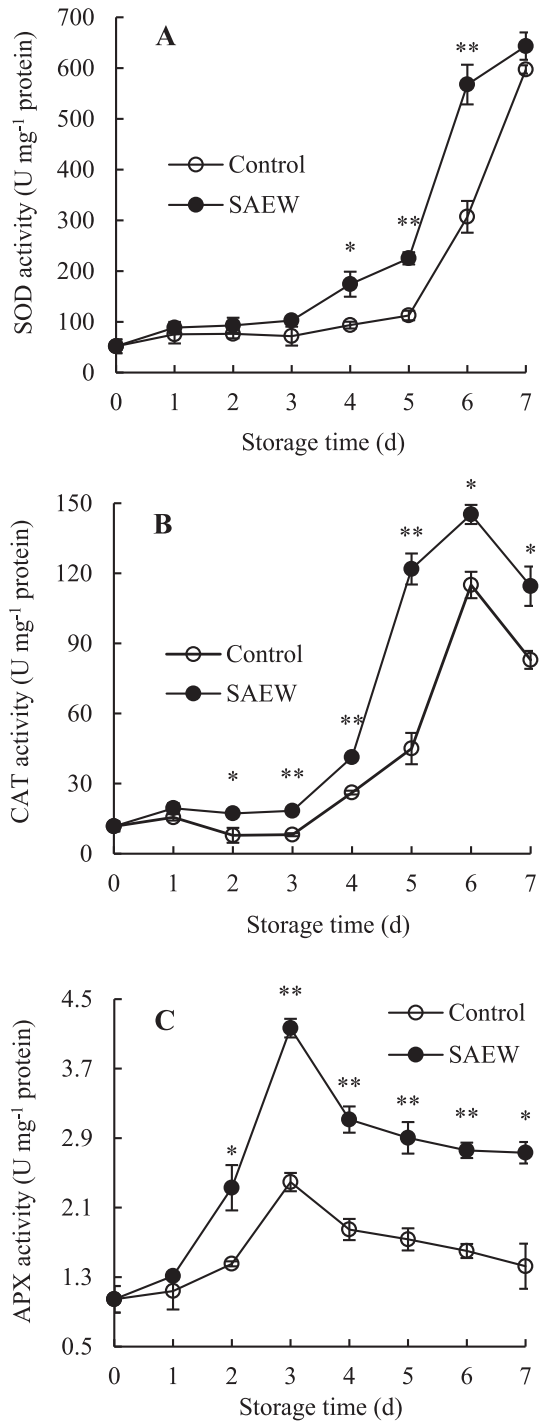

Fig. 5. Effects of SAEW treatment on the SOD activity (A), CAT activity (B) and APX activity (C) in pericarp of litchi fruit during storage. The data in the figures were expressed as the mean ± standard error (n = 3). The symbol * and ** in the figures respectively indicated significant differences ($p < 0.05$) and highly significant differences ($p < 0.01$) between the SAEW-treated litchis and the control litchis on the same day of storage, according to one-way ANOVA and independent sample *t*-test. ○, Control litchis; ●, SAEW-treated litchis. ANOVA, analysis of variance; SAEW, slightly acidic electrolyzed water; SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase.

APX activity in pericarp of the control litchi fruit enhanced slightly within 0–3 d of storage, while dropped gradually during storage days 3–7 (Fig. 5C). SAEW-treated fruit exhibited higher APX activity than the control fruit during storage, with significant differences between days 2 and 7. For example, on day 7, SAEW-treated fruit presented 92% higher level of APX activity than the control fruit.

3.6. Pericarp non-enzymatic antioxidant abilities

During storage, a gradually decreasing content of GSH was observed in the control group (Fig. 6A). A slower declination of GSH level was displayed in SAEW-treated fruit than the control fruit. In detail, during

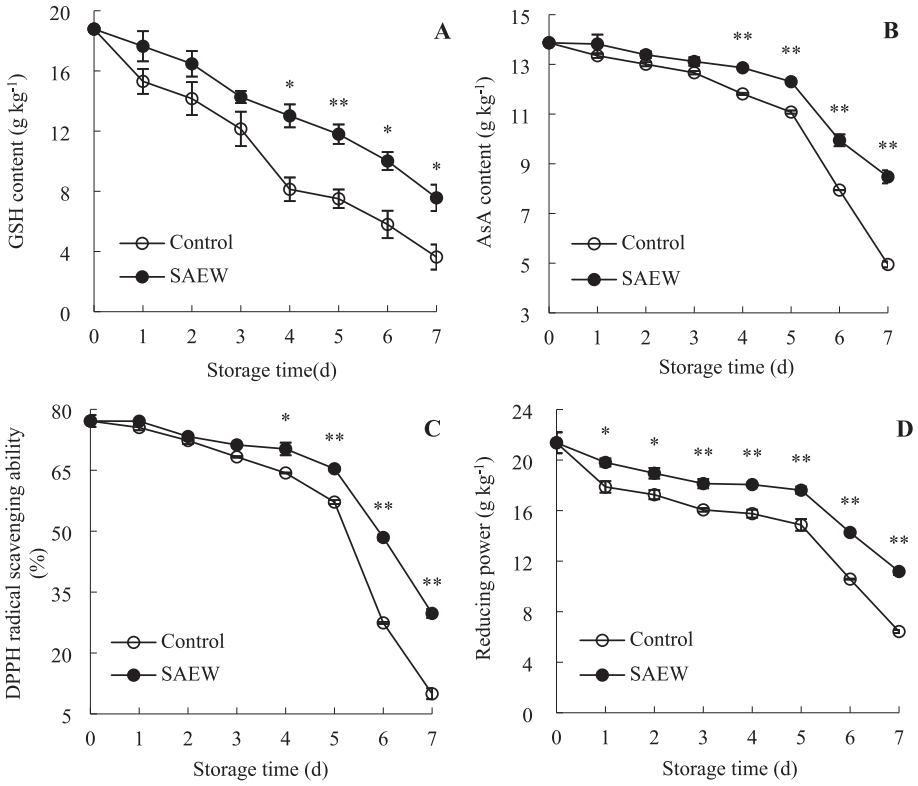

Fig. 6. Effects of SAEW treatment on the GSH content (A), AsA content (B), DPPH radical scavenging ability (C) and reducing power (D) in pericarp of litchi fruit during storage. The data in the figures were expressed as the mean ± standard error (n = 3). The symbol * and ** in the figures respectively indicated significant differences ($p < 0.05$) and highly significant differences ($p < 0.01$) between the SAEW-treated litchis and the control litchis on the same day of storage, according to one-way ANOVA and independent sample *t*-test. ○, Control litchis; ●, SAEW-treated litchis. ANOVA, analysis of variance; SAEW, slightly acidic electrolyzed water; GSH, glutathione; AsA, ascorbic acid; DPPH, 1, 1-diphenyl-2-picrylhydrazyl.

storage, SAEW-treated fruit exhibited higher level of GSH, with 60%, 57%, 72%, and 108% higher GSH level on day 4, day 5, day 6, and day 7, respectively, as compared to the control fruit (Fig. 6A).

AsA content, DPPH radical scavenging ability, and reducing power in litchi pericarp declined slightly over the initial 5 d of storage, and then dropped rapidly afterward (Fig. 6B-6D). It was worth noting that, compared to the control fruit, SAEW treatment retarded the declines of AsA content, DPPH radical scavenging ability, and reducing power in pericarp of litchi fruit during storage. For example, on day 7, 71%, 199%, and 74% higher levels of these three indices were shown in SAEW-treated fruit than the control fruit, respectively.

4. Discussion

4.1. SAEW treatment induced the changes in the indices of the quality and storability of postharvest litchis

Fresh litchi fruit will rapidly undergo pericarp browning under normal temperature storage conditions, leading to fruit decay and quality deterioration, resulting in the loss of flavor, nutrition, and the attractive red color (Marak et al., 2023). Present work indicated SAEW treatment for litchi fruit could effectively reduce pericarp browning and fruit weight loss, and sustain higher rate of commercially acceptable fruit (Fig. 1). The work of Zhang, Liu, et al. (2023) also showed similar results, indicating that SAEW treatment could restrain pericarp browning and reduce weight loss of carambola fruit during storage at 18 °C.

The chromaticity system is used to objectively measure the color response to the changes in fruit color (Beyaz et al., 2010). Also, the degradation of chlorophyll and anthocyanins could influence the color of fruits (Chen et al., 2023). In this study, for the control litchi fruit, with the increasing storage time, litchi's pericarp browning index elevated, while the values of pericarp chromaticity (L^*, a^*, b^*, C) (Fig. 2A-2D) and the contents of pericarp pigment (chlorophyll and anthocyanin) (Fig. 2E, F) decreased. Further analyses revealed that the elevated index of pericarp browning (Fig. 1A) was negatively correlated with the reduced values of pericarp chromaticity (Fig. 2A-2D) and the reduced contents of pericarp pigment (Fig. 2E, F), indicating the development of litchi pericarp browning was associated with the reduced values of pericarp chromaticity (L^*, a^*, b^*, C) and the dropped contents of pericarp pigment (chlorophyll and anthocyanin). Additionally, compared to the control litchi fruit, SAEW treatment could effectively reduce litchi pericarp browning (Fig. 1A, B), retain higher values of pericarp chromaticity (L^*, a^*, b^*, C) and pericarp pigment (chlorophyll and anthocyanin), implying SAEW-inhibiting litchi pericarp browning was related to the alleviated declines of pericarp chromaticity (L^*, a^*, b^*, C) and pericarp pigment (chlorophyll and anthocyanin). Postharvest treatments, such as chitosan (Kumar, Neeraj, & Singla, 2020), hydrogen sulfide (Siddiqui et al., 2021), or methyl jasmonate (Deshi et al., 2021), also showed a similar suppression of litchi pericarp browning and retained the pericarp color through reducing the degradation of pericarp pigment.

TSS, total soluble sugar, sucrose, reducing sugar, TA, and vitamin C are crucial metrics to assess the nutritional value of fruits (Chen et al., 2020). In this study, for the control litchi fruit, TSS (Fig. 3A) and reducing sugar (Fig. 3D) contents demonstrated a tendency of initially rising and then falling during storage. The transient rise in TSS content during the early storage stage was due to the complicated polysaccharide hydrolysis and pectin change in the pulp (Kumar et al., 2020). Subsequently, due to the respiratory consumption and the degradation of macromolecular carbohydrates during storage, TSS content gradually decreased (Wu et al., 2017). The increasing trend of reducing sugar content during the early storage period (0–3 d) was due

to the decomposition of sucrose into reducing sugar (glucose and fructose) in litchis, which causing a decrease in sucrose content (Fig. 3C), and an increase in reducing sugar content (Chen et al., 2020). In the later stage of storage, monosaccharides such as glucose and fructose were consumed as respiratory substrates in the respiratory metabolic pathways, leading to a decrease in reducing sugar content (Chen et al., 2020). Additionally, the contents of total soluble sugar (Fig. 3B), sucrose (Fig. 3C), and vitamin C (Fig. 3F) decreased with the extension of storage time, which were caused by the fruit respiration. Furthermore, the decrease in TA content (Fig. 3E) during the early storage period (0–4 d) was related to the consumption of TA as respiratory substrate. However, the increase in TA content (Fig. 3E) during the later stage of storage (4–7 d) was associated with *Peronophthora litchii*-induced litchi downy blight disease and *Geotrichum candidum*-induced litchi sour-rot (Jiang et al., 2018a, 2018b; Lin, Liu, Lin, Chen, & Kong, 2009).

Moreover, compared to the control litchis, throughout the entire storage period, SAEW treatment maintained higher contents of TSS, total soluble sugar, sucrose, and vitamin C (Fig. 3A-3C, 3F), and lowered the content of reducing sugar (Fig. 3D). Moreover, SAEW treatment displayed higher content of TA during the early storage period (1–4 d), but showed lower level of TA content during the later storage period (5–7 d). These data indicated that SAEW treatment could effectively maintain the quality of postharvest litchis.

4.2. ROS metabolism participates in SAEW-enhancing the quality attributes and the storability of postharvest litchis

4.2.1. The levels of ROS and MDA

ROS includes $O_2^{\cdot-}$, H_2O_2, and ·OH (Lin et al., 2021; Lin, Chen, et al., 2020). Under normal circumstances, the production and elimination of ROS in fresh fruit could establish a relative equilibrium to protect fruit tissue from the attack of an excessive accumulation of ROS (Bai et al., 2022; Lin et al., 2022; Lin, Chen, et al., 2020). However, with the extension of storage time, the capacity of scavenging ROS is less than the ability of generating ROS (Lin, Chen, et al., 2020; Tang et al., 2020), which lead to the disrupted balance between ROS production and ROS elimination, thereby resulting in an excessive accumulation of ROS (Huang et al., 2022; Yu et al., 2022). MDA is a production of cell membrane lipid peroxidation (Lin et al., 2014). The level of MDA is related to the damage degree of cell membrane integrity and the quality deterioration in postharvest fruit (Huang et al., 2022; Lin et al., 2021).

Some works have shown that, with the extended storage time, the massive accumulation of ROS attacked the cell structure, leading to the oxidative damage to cells and the peroxidation of cell membrane lipid, and ultimately causing the deterioration of fruit quality (Hou, Li, Zheng, & Jin, 2021; Sun et al., 2023; Xue et al., 2020). Dong et al. (2022) found that, during storage, the increased contents of H_2O_2 and MDA led to the reduced quality and storability of *Rosa roxburghii* fruit, manifested by an increase in decay incidence and weight loss, as well as a decrease in fruit firmness, TSS, and TA. The work of Hou et al. (2021) also showed similar trends, indicating that an elevated index of pericarp browning in loquat fruit was due to the declined capacity of scavenging ROS and the elevated accumulation of ROS. However, sustaining a balanced ROS metabolic state helps reduce ROS production and accumulation, and contributes to maintaining the quality of postharvest fruits (Jia, Li, Liu, & He, 2022; Yuan et al., 2023). Jia et al. (2022) revealed that the treatment of acidic electrolyzed water (AEW) for jujube fruit could reduce fruit disease index, which was related to the lower $O_2^{\cdot-}$ production rate, and the lower levels of H_2O_2 and MDA. Yuan et al. (2023) indicated that 1-methylcyclopropene (1-MCP) treatment lowered $O_2^{\cdot-}$ generation rate, reduced the levels of H_2O_2, MDA, and the peroxidation of membrane lipid, thereby retaining the quality attributes of mango fruit during storage.

In this research, the $O_2^{\cdot-}$ production rate (Fig. 4A), H_2O_2 level (Fig. 4B), and MDA level (Fig. 4C) in the control group increased with the extension of storage time. The raised pericarp browning index (Fig. 1A) was positively correlated with the elevated levels of H_2O_2 and MDA (Fig. 4B, C), with the correlation coefficients (r) 0.943** and 0.971**, respectively. These data indicated that the browning of litchi pericarp was closely correlation with the increased generation and accumulation of ROS, and the elevated peroxidation of cell membrane lipid.

Additionally, compared to the control litchis, during storage, SAEW treatment presented lower levels of pericarp browning index (Fig. 1A), H_2O_2 and MDA (Fig. 4B, C), implying that SAEW-reducing litchi pericarp browning was due to the lower ROS generation and accumulation, and the reduced peroxidation of cell membrane lipid.

4.2.2. Activities of ROS scavenging enzymes

ROS scavenging enzymes is a typical strategy for removing ROS to prevent an excessive accumulation of ROS and the oxidative stress, thus keeping the redox equilibrium in fresh fruit (Tang et al., 2020; Xue et al., 2020).

ROS scavenging enzymes consist of SOD, CAT, and APX (Javed et al., 2023; Yu et al., 2022). Among them, SOD acts as the frontline defense, playing a critical role in clearing ROS (Lin et al., 2022; Liu et al., 2022; Sun et al., 2023). SOD is responsible for converting $O_2^{\cdot-}$ into O_2 and H_2O_2 (Yu et al., 2022). APX and CAT are responsible for scavenging H_2O_2, which catalyze the dissociation of H_2O_2 into H_2O and O_2, and effectively reduce the potential oxidative damage to cells (Lin et al., 2014; Sun et al., 2023).

Previous works indicate that, the maintenance of quality attributes and the improved storability of fresh produces are related to the higher activities of ROS scavenging enzymes and the lower level of ROS (Jia et al., 2022; Lai et al., 2020; Yuan et al., 2023; Zhang, Shan, et al., 2023). Jia et al. (2022) found that AEW treatment for jujube fruit presented higher activities of SOD and CAT, lower levels of $O_2^{\cdot-}$ production rate and H_2O_2, and lower index of fruit disease. Yuan et al. (2023) revealed that the treatment of 1-MCP-improved the quality attributes of mango fruit was due to the higher activities of SOD, CAT, and APX, a lower generation rate of $O_2^{\cdot-}$, and a lower H_2O_2 content. Lai et al. (2020) also revealed that *Photorhabdus luminescens* Hb1029 could significantly improve the quality of litchis, maintaining a 20% higher TSS content and a 165% lower index of pericarp browning as compared to the control group on day 7, which was related to the enhanced activities of CAT and SOD, and the reduced accumulation of H_2O_2.

In this work, compared to the control litchis, during storage, SAEW-treated litchis exhibited lower pericarp browning index (Fig. 1A), higher rate of commercially acceptable fruit (Fig. 1C), higher activities of SOD, CAT, and APX (Fig. 5), lower levels of $O_2^{\cdot-}$ production rate (Fig. 4A), H_2O_2 (Fig. 4B), and MDA (Fig. 4C). These data indicate that the application of SAEW to litchi fruit resulted in the enhanced storability, which was due to SAEW-induced higher activities of ROS scavenging enzymes to scavenge ROS and lead to a low level of ROS, thereby alleviating the peroxidation of cell membrane lipid, and maintaining the structure and function of cells in postharvest litchi fruit.

4.2.3. Non-enzymatic antioxidant abilities

AsA and GSH, which are the main non-enzymatic endogenous antioxidant compounds, participate in GSH-AsA cycle (Jiang et al., 2018b). AsA can oxidize H_2O_2 to H_2O, thereby reduce ROS accumulation and prevent cell oxidative damage (Lin, Chen, et al., 2020). During this process, AsA can be oxidized to dehydroascorbate (DHA), which is then reduced to AsA by GSH, protecting cellular components from oxidative stress by maintaining adequate AsA level (Tang et al., 2020; Xue et al., 2020). Moreover, DPPH radical scavenging ability and reducing power are the main indicators to evaluate the antioxidant capacity of fruits (Jiang et al., 2018b; Lin, Chen, et al., 2020).

Some works suggest that the levels of AsA, GSH, DPPH radical scavenging ability, and reducing power are related to the ROS level, the quality attributes and the storability of fresh produces (Lin et al., 2014; Lin et al., 2021; Lin, Chen, et al., 2020; Lin, Lin, et al., 2020). Lin et al.

(2014) indicated that, during the development of longan pericarp browning, the levels of AsA and GSH reduced, while the generation rate of $O_2^{\cdot-}$ increased. Lin et al. (2021) also found that H_2O_2-induced the pulp breakdown of fresh longan was due to the higher level of generation rate of $O_2^{\cdot-}$, and the lower levels of AsA, GSH, DPPH radical scavenging ability, and reducing power. However, the applications of chitosan (Lin, Chen, et al., 2020), propyl gallate (Lin, Lin, et al., 2020), or ATP (Lin et al., 2022) could effectively suppress the pulp breakdown of fresh longan, which were related to the lower level of ROS, and the higher levels of AsA, GSH, DPPH radical scavenging ability, and reducing power. Kumar et al. (2020) also suggested that chitosan could greatly lower the weight loss percentage, TSS, TA, and browning (chromaticity value of L^*, a^*, b^*) in litchi fruit during storage by preserving a high level of antioxidant activity.

In this work, for the control fruit, during the development of litchi pericarp browning (Fig. 1A, B), the GSH content (Fig. 6A), AsA content (Fig. 6B), capability to scavenge DPPH radical (Fig. 6C), and reducing ability (Fig. 6D) decreased, while H_2O_2 content (Fig. 4B), and MDA content (Fig. 4C) increased. Further analyses revealed that, during the storage period, a negative relationship was derived between the escalated pericarp browning index (Fig. 1A) and the reduced GSH content, AsA content, capability to scavenge DPPH radical, and reducing ability (Fig. 6A-6D), with r of −0.865**, −0.985**, −0.986**, and − 0.971**, respectively. Furthermore, a negative relationship was also obtained between the elevated H_2O_2 level (Fig. 4B) and the reduced GSH content, AsA content, capability to scavenge DPPH radical, and reducing ability (Fig. 6A-4D), with r of −0.938**, −0.926**, −0.925**, and − 0.911**, respectively. Similarly, the elevated MDA content (Fig. 4C) showed a negative correlation with GSH level (r = −0.899*), AsA level (r = −0.955**), reducing ability (r = −0.947**), and capability to scavenge DPPH radical (r = −0.958**). These data indicated that, the increases in litchi pericarp browning index (Fig. 1A), H_2O_2 content (Fig. 4B), and MDA content (Fig. 4C) were resulted from a reduction in the ability to scavenge ROS, which accelerated the peroxidation of cell membrane lipid and the disruption of cell structure in litchi fruit. Meanwhile, with the occurrence of these unfavorable activities, the quality and storability properties in the control litchi fruit, including the commercially acceptable fruit rate (Fig. 1C), the pericarp chromaticity values (L^*, a^*, b^*, and C) (Fig. 2A-2D), the contents of pericarp chlorophyll and anthocyanin (Fig. 2E, F), the levels of pulp total soluble sugar (Fig. 3B), sucrose (Fig. 3C), and vitamin C (Fig. 3F), displayed a decreasing tendency, while the pericarp browning index (Fig. 1A) and the weight loss percentage (Fig. 1D) presented a climbing tendency. Therefore, the quality decline of postharvest litchi fruit was associated with the decreased ability of scavenging ROS and an excessive accumulation of ROS.

Additionally, compared to the control litchis, during storage, SAEW-treated litchis presented higher levels of AsA, GSH, DPPH radical scavenging ability, and reducing power (Fig. 6A-6D), lower levels of $O_2^{\cdot-}$ production rate, H_2O_2, and MDA (Fig. 4A-4C). SAEW-treated litchis

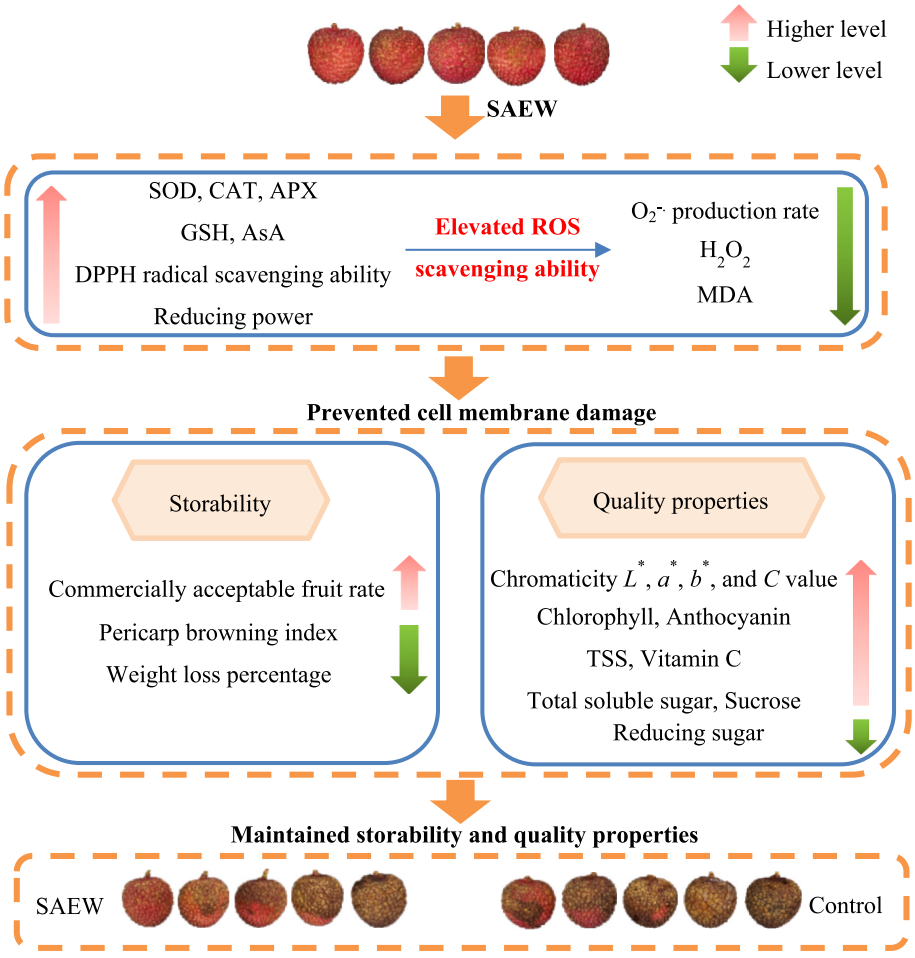

Fig. 7. The possible mechanism of SAEW enhancing the quality attributes and the storability of postharvest litchis through regulating ROS metabolism. The up arrow (↑) in the figure represented the higher levels of indices in the SAEW-treated litchis as compared to the control litchis; while the down arrow (↓) in the figure represented the lower levels of indices in the SAEW-treated litchis as compared to the control litchis. SAEW, slightly acidic electrolyzed water; ROS, reactive oxygen species; SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase; GSH, glutathione; AsA, ascorbic acid; DPPH, 1, 1-diphenyl-2-picrylhydrazyl; $O_2^{\cdot-}$, superoxide anion radical; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; TSS, total soluble solid.

improve the quality and storability characteristics, including higher rate of commercially acceptable fruit (Fig. 1C), higher pericarp chromaticity values (L^*, a^*, b^*, and C) (Fig. 2A-2D), higher contents of pericarp chlorophyll and anthocyanin (Fig. 2E, F), higher levels of pulp TSS, total soluble sugar, sucrose (Fig. 3A-3C), and vitamin C (Fig. 3F), while lower levels of pericarp browning index (Fig. 1A) and weight loss percentage (Fig. 1D). These data suggested that SAEW-induced higher quality and storability of postharvest litchis was closely correlation with the increased antioxidant activity, the reduced ROS level, and the alleviated peroxidation of cell membrane lipid.

Based on the aforementioned results, the potential mechanisms of SAEW enhancing the quality attributes and the storability of postharvest litchis through regulating ROS metabolism were illustrated in Fig. 7.

5. Conclusions

This work found that the quality and storability characteristics of litchi fruit could be significantly elevated by SAEW (40 mg L^{-1} ACC) through regulating ROS metabolism. In particular, the SAEW treatment demonstrated the ability to inhibit the excessive generation of $O_2^{\cdot-}$, H_2O_2, and MDA by boosting APX, CAT, and SOD activities. Besides, it helped maintain higher capability to scavenge DPPH radical and reducing power, as well as keep the elevated levels of GSH and AsA. As a result, SAEW treatment alleviated lipid peroxidation, preserved the cellular function and structural integrity, and ultimately contributed to the overall improvement in the quality properties of litchis. These findings suggested that SAEW treatment held a great potential as an effective and safe approach for enhancing the storability of litchi fruit during storage. Nonetheless, the molecular mechanism by which SAEW delayed litchi pericarp browning and maintained fruit quality in relation to ROS metabolism remain unclear. Consequently, future research should focus on the metabolomics and transcriptomics to better understand their molecular mechanism.

CRediT authorship contribution statement

Jing Zhang: Writing – original draft, Supervision, Formal analysis, Data curation. **Xuezhen Chen:** Writing – original draft, Investigation, Formal analysis, Data curation. **Qingqing Liu:** Investigation. **Meiling Li:** Investigation. **Shujuan Feng:** Investigation. **Mingyu Lin:** Investigation. **Yihui Chen:** Writing – review & editing, Supervision, Project administration. **Hetong Lin:** Writing – review & editing, Supervision.

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

The authors do not have permission to share data.

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