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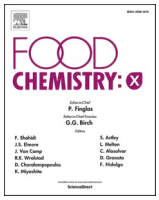

The effects of aurone on the yellowing of fresh-cut water chestnuts

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ABSTRACT

Yellowing is the main reason for deterioration of edible quality of fresh cut water chestnuts (FCWCs). The mechanism of aurone inhibiting the yellowing of FCWCs was studied. FCWCs were treated with aurone (0.2, 0.6 and 1.0 %). The controls yellowed completely on day 9. The treatment sample with 1.0 % aurone did not yellow on day 9. Compared to the controls, aurone (1.0 %) completely inhibited the production of eriodictyol during 9 d of storage. Aurone (1.0 %) reduced peroxidase activity of FCWCs by 23 % on day 9. The effects of aurone on naringenin concentration, polyphenol oxidase activity, phenylalanine lyase activity, number of thermophilic bacteria colonies, and number of yeasts and molds colonies of FCWCS were not significant. Aurone reduced the yellowing by decreasing the yield of eriodictyol and inhibiting POD activity. Aurone (1.0 %) can be used to inhibit the yellowing of FCWCs in practice.

Introduction

Water chestnuts (WCs) are underground stems of a kind of sedge plant. WCs are rich in nutrient substances, such as 1.2 % protein, 14.2 % carbohydrate, 1.1 % dietary fiber, 0.02 % carotenes, 0.000003 % vitamin A, 0.00002 % vitamin B, and 0.00065 % vitamin E. WCs can prevent some chronic diseases and detoxify and protect the kidney. Recently, scientists extracted an antibacterial substance from WCs. The antibacterial substance was called puchiins, which can inhibit the growth of some microorganisms. WCs have excessive pathogens on their skin because they grow underground. Peeling can remove most pathogens. Fresh-cut water chestnuts (FCWCs) taste sweeter and are convenient to eat. Therefore, people prefer to eat FCWCs.

The surface of FCWCs yellowed during storage. The yellowing reduces the acceptability of FCWCs, shortens their shelf life, and lowers their commercial value. At first, the color change was considered to be enzymatic browning, which was catalyzed by polyphenol oxidase (PPO). Some scientists have conducted a lot of researches to curb the color change of FCWCs. Enzymatic browning of FCWCs was inhibited by chitosan film (Pen & Jiang, 2003). They used 5 % acetic acid to help chitosan dissolve in water. Enzymatic browning of FCWCs was inhibited by citric acid (Jiang et al., 2004). Peng and Jiang inhibited the browning of FCWCs by exogenous salicylic acid (Jiang et al., 2004). FCWCs soaked in these highly acidic solutions might be too acidic to eat. Peng adopted H_2O_2 to curb the enzymatic browning of FCWCs (Peng et al., 2008). H_2O_2 had a high residue in FCWCs, leaving a negative impact on human health (Peng et al., 2008). Pure N_2 was employed to inhibit the browning of FCWCs (You et al., 2012). Anaerobic respiration occurred in FCWCs, and alcohol was produced under short-term anoxia and reduced the edible quality of FCWCs (You et al., 2012). Teng et al. and Zhu et al. investigated the effect of eugenol emulsions on the browning of FCWCs (Teng et al., 2020; Zhu et al., 2022). Eugenol is expensive and difficult to extract (Teng et al., 2020; Zhu et al., 2022). Browning of FCWC was reduced by high-pressure CO_2 (Kong et al., 2021). High pressure might damage tissues of FCWCs easily (Kong et al., 2021). These researches have suggested that peroxidase (POD) and phenylalanine lyase (PAL) also catalyzed the browning of FCWCs (Teng et al., 2020; Zhu et al., 2022). Recently, some researchers proposed that the browning of FCWCs was a kind of yellowing (Pan et al., 2015; Song et al., 2019b). The yellowing substances were isolated and identified as eriodictyol and naringenin (Pan et al., 2015). Ferulic acid and ascorbic acid were used to inhibit the yellowing of FCWCs (Song et al., 2019b). Ascorbic acid could not inhibit the yellowing. Ferulic acid made FCWCs sour. Hydrogen-rich water was adopted to reduce the yellowing of FCWCs (Li et al., 2022). Its effect was not ideal.

Aurone (AU) is a colorless bioactive substance extracted from plants.

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It has no taste and is soluble in water. It has no negative effects on human health and the environments (Nabi et al., 2022). To date, there is no report on aurone applied in food industry. Aurone was adopted to inhibit the yellowing of FCWCs in the study. The objective of this study was to elucidate the mechanism of AU inhibiting the yellowing of FCWCs. The results of the study will provide a theoretical basis for the application of AU in inhibiting the yellowing of FCWCs.

Material and methods

Material

Aurone was purchased from Sinopharm Chemical Reagent Co. ltd, Beijing, China and analytical reagent. WCs were obtained from Huangshi, China and transported to our lab immediately. A similar size WCs were selected roughly. Any putrid or broken WCs were removed. All reagents were analytically pure.

FCWCs preparation

WCs were peeled and cut into 2–3 mm slices with a stainless steely knife. These slices were sterilized with O_3 for 30 min and then were left to drain naturally in a plastic basket for 10 min. These sterilized slices were FCWCs.

Experimental design

FCWCs (3.5 ± 0.1 kg) were immersed in 35.0 ± 0.1 L of aurone aqueous solution for 1 h. Aurone concentration were 0.2, 0.6, and 1.0 % (m m^{-1}), respectively. FCWCs were left to drain naturally in a plastic basket for 10 min. Aurone solution was replaced with H_2O in the controls. FCWCs were filled into plastic crispers (15.5 cm × 8.5 cm × 7.5 cm) with lids. FCWCs (10 g) were filled with every crisper. These crispers filled with FCWCs were deposited at 90–93 % RH and 1 ℃.

Analyzing yellowing degree

Yellowing degree (YD) was used to quantify the yellowing of FCWCs. YD of FCWCs was evaluated by b*. The b* value represented the change in color from blue to yellow. Eighteen crispers of each sample were fixed to measure b*. The measurement was performed every 3 d, and six repetitions were carried out each time.

Every repetition adopted three crispers. Measurement of b* referred to the method of Qiao et al. (2021) and was performed with a portable colorimeter (NH300, Shenzhen 3NH Technology CO., ltd, China). YD was calculated according to Formula 1, with the unit of %.

$$YD = \frac{b_t^* - b_0^*}{b_0^*} * 100\% \quad (1)$$

where b_0* and b_t* denote b* of day 0 and t, respectively.

Analyzing eriodictyol and naringenin concentrations

Eriodictyol and naringenin were the products of the yellowing. Eriodictyol and naringenin concentrations were used to determine the yield of yellowing substants. Eighteen crispers were randomly selected to measure eriodictyol and naringenin concentrations every 3 d. Six repetitions were performed each, with every repetition using three crispers.

Eriodictyol and naringenin concentrations were measured by the method of Song et al. (2019a) and made a minor modification. A highly performance liquid chromatography (HPLC) (Essentia LC-15C, Shimadzu Corporation, Japan) was used. FCWCs (30 g) were homogenized in absolute ethyl alcohol (m v^{-1} = 1 1^{-1}) with a blender (C93T, Joyoung Co., ltd., China). The homogeneous liquid was broken for 30 min with an ultrasonic cell muller (JRA-650E, Xinzhi Co., ltd., China), and then stirred for 30 min with a digital agitator (RW20, IKA Co., ltd., German). The mixture was centrifuged at 12,000×*g* for 20 min at 25 ℃ with a centrifuge (Allegra X-12, Beckman, USA). The residue was re-washed with absolute ethyl alcohol and centrifuged again at 12,000×*g* for 20 min. The combined supernatants were placed into a rotary evaporator to remove ethyl alcohol at 48 ℃ under vacuum. The concentrate was dissolved in 4 mL HPLC grade methanol and used to do HPLC analyses after passing through a 0.22 μm Millipore filter. The mobile phases consisted of ultrapure water (eluent A) and HPLC grade methanol (eluent B). The injection volume was 10 μL. The flow rate of the mobile phase was 1.0 mL min^{-1}. The chromatographic column was a Inert-Sustain C18 column (4.6 × 250 mm, 5 μm), which operating temperature was 30 ℃. The gradient elution program was as follow: 0–10 min, 10 %–20 % B; 10–15 min, 20 % − 30 % B; 15–25 min, 30 %–40 % B; 25 – 35 min, 40 %–50 % B. Eriodictyol and naringenin concentrations were achieved by comparing each peak retention time and peak area with those of the standard.

Analyzing enzyme activities

PPO, POD and PAL might be the enzymes that catalyze the yellowing of FCWCs. PPO, POD and PAL activities were measured to determine whether they catalyzed the yellowing.

PPO and POD activities

Eighteen crispers used to analyze PPO and POD activities were randomly selected every 3 d. Six repetitions were performed each, with every repetition applying three crispers.

Extracting PPO and POD crude enzyme solutions referred to the method of Camiscia et al. (2020). FCWCs (30 g) were homogenized in 40 mL of mixed solution (0.05 mol/L phosphate buffer (pH 6.9), 35 mL; 5 % polyvinylpyrrolidone (PVP), 5 mL) with a blender (C93T, Joyoung Co., ltd., China). The homogeneous liquid was broken for 10 min with an ultrasonic cell muller (JRA-650E, Xinzhi Co., ltd., China), and then stirred for 30 min with a digital agitator (RW20, IKA Co., ltd., German). The mixed liquid was filtered through four layers of cotton cloth. The filtrate was filled in 2 mL centrifuge tubes. These filtrates were centrifuged at 12,000×*g* for 30 min at 4 ℃ in a centrifuge (Allegra X-12, Beckman, USA). All supernatant, which were the crude enzyme solution of PPO and POD, were collected.

Analyzing PPO activity was based on the method of Yuzugullu Karakus et al. (2021). A mixture solution (0.05 mol/L phosphate buffer (pH 6.9), 1.8 mL; 0.1 mol/L 4-methylcatechin, 1 mL; PPO crude enzyme solution, 0.2 mL) was employed to measure the absorbance with a visible spectrophotometer (5100, Yiheng Instrument Co. ltd., Beijing, China). The wavelength was 420 nm, the absorbance was recorded every 10 s, and the measurement lasted for 3 min. The PPO crude enzyme solution was replaced with phosphate buffer in the controls. The number of PPO required to increase the absorbance by 0.001 per kilogram FCWCs per second was defined as a unit of PPO activity (U s^{-1} kg^{-1}).

POD activity was analyzed using the method of de Oliveira et al. (2021). A mixture solution (0.05 mol/L phosphate buffer (pH 6.9), 2.6 mL; 5 % H_2O_2, 0.2 mL; POD crude enzyme solution, 0.2 mL) was employed to measure the absorbance with a visible spectrophotometer (5100, Yiheng Instrument Co. ltd., Beijing, China). The wavelength was 470 nm, the absorbance was recorded every 10 s, and the measurement lasted for 3 min. The POD crude enzyme solution was replaced with phosphate buffer in the controls. The number of POD required to increase the absorbance by 0.001 per kilogram FCWCs per second was defined as a unit of POD activity (U s^{-1} kg^{-1}).

PAL activity

Eighteen crispers used to analyze PAL activity were randomly selected every 3 d. Six repetitions were performed each, with every repetition applying three crispers.

PAL crude enzyme solution was extracted by the method of Rahmatabadi et al. (2019). FCWCs (30 g) were homogenized in 40 mL of mixed solution (0.2 mol/L sodium borate buffer, 35 mL; 5 % PVP, 5 mL) with a blender (C93T, Joyoung Co., ltd., China). The homogeneous liquid was broken for 10 min with an ultrasonic cell muller (JRA-650E, Xinzhi Co., ltd., China), and then stirred for 30 min with a digital agitator (RW20, IKA Co., ltd., German). The mixed liquid was filtered through four layers of cotton cloth. The filtrate was filled in 2 mL centrifuge tubes. These filtrates were centrifuged at 12,000×*g* for 30 min at 4 ℃ in a centrifuge (Allegra X-12, Beckman, USA). All supernatant, which were the crude enzyme solution of PAL, were collected.

PAL activity was investigated using the method of Li et al. (2018). A mixed solution (0.5 mol/L Na_2B4O_7 buffer, 2.6 mL; 0.5 mol/L L-phenylalanine, 0.2 mL; PAL crude enzyme solution, 0.2 mL) was heated in a water bath of 35 ℃ for 1 h. The reaction of the mixed solution was stopped by 0.5 mL of 35 % trichloroacetic acid. The absorbance of the mixed solution was measured with an ultraviolet spectrophotometer (7100, Yiheng Instrument Co. ltd., Beijing, China). The wavelength was 290 nm, the PAL crude enzyme solution was replaced with phosphate buffer in the controls, and the absorbance was recorded every 10 min. The measurement lasted for 1 h. The number of PAL required to increase the absorbance by 0.001 per kilogram FCWCs per second was defined as a unit of PAL activity (U s^{-1} kg^{-1}).

Analyzing microbial colonies

Number of thermophilic bacteria colonies and number of yeasts and molds colonies were used to determine whether the microbial populations exceeded the safe level. Eighteen crispers were randomly selected to analyze microbial colonies every 6 d. Six repetitions were performed each, with every repetition applying three crispers.

The analysis of microbial colonies was conducted based on the method of Bata Gouda et al. (2021). All glassware was sterilized for 2 h at 165 ℃ with a drying oven (DO-9070, Jingmi Scientific Instrument Co., ltd, Shanghai, China). Thermophilic counting medium and yeasts and molds counting medium were sterilized by U03 model with an autoclave (GR60, Xiamen Zhiwei Instrument Co., ltd, China). FCWCs (30 g) and 270 mL of sterile H_2O were homogenized with a sterile homogenizer (VOSHIN-400R, Jintan Co., ltd., China) for 10 min. The homogeneous liquid was diluted from 10^{-2} to 10^{-4}. The medium (15–20 mL) was evenly spread out in the culture dish (diameter, 9 cm). The suspension (0.2 mL) was evenly distributed on the medium with a 0.5 mL pipette. Parallel tests were performed in 3 culture dishes for each dilution. These operations were completed on a clean bench a clean bench (SW-CJ-2F, Shanghai Precision Instrument Co., ltd, China). Yeasts and molds were cultured for 3 d at 25 ℃. The thermophilic bacteria were cultured for 2 d at 35 ℃. The number of colonies were counted. Unit of the number of colonies was lg CFU g^{-1}.

Analyzing the suppression type of aurone

In order to determine how aurone inhibited POD activity, the Lineweaver-Burk plot, the Slope-Inhibitor plot, and the Intercept-Inhibitor plot were applied. POD concentration was 0.01 %. Aurone concentrations were 0, 0.2, 0.4, 0.6, 0.8, and 1.0 %, respectively. The POD substrate was guaiacol, which concentrations were 0.01, 0.02, 0.03, 0.04, and 0.05 %, respectively. K_i and K_{is} were calculated according to formulas 2 and 3 to further determine how aurone was combined.

$$K_i = \frac{K_m{}^*[I]}{V_{max}{}^*(Slope - \frac{K_m}{V_{max}})} \quad (2)$$

$$K_{is} = \frac{[I]}{V_{max}{}^* Y_{intercept} - 1} \quad (3)$$

where V_{max} denotes the maximum reaction rate; K_m represents the Michaelis-Menten constant; K_i refers to the free enzyme inhibition constant; K_{is} stands for the bound enzyme inhibition constant. $Y_{intercept}$ designates the intercept of Y coordinate. [I] denotes the concentrations of the inhibitors.

Statistical analysis

All analyses were conducted with 6 parallel trials and reported as mean ± standard deviation (SD) (n = 6, each parallel trial used 3 crispers). Tukey test at $p < 0.05$ was performed with Origin 8.5.

Results

Yd

Photographs of FCWC of the controls and the treatment samples with 0.2, 0.6, and 1.0 % aurone were illustrated in Fig. 1. No yellowing appeared in the aurone treatment samples and the controls on day 0 and 3, respectively. The treatment sample with 0.2 % aurone and the controls began to yellow on day 6, and yellowed completely on day 9. The treatment sample with 0.6 % aurone began to yellow on day 9. The treatment sample with 1.0 % aurone did not yellow during storage. The controls and the treated samples underwent the same treatment except that the concentration of AU was different. Therefore, the different color changes of the controls and the treated samples during storage were caused by the treatment of the different concentrations of AU.

% AU during storage.

The results of yellowing degrees (YDs) of FCWCs of the controls and the treatment samples with 0.2, 0.6, and 1.0 % aurone were exhibited in Fig. 2A. YDs of the controls were 0.83, 23.3, and 95.65 % on day 3, 6, and 9, respectively. YDs of the treatment sample with 0.2 % aurone were 0.82, 22.43, and 95.05 % on day 3, 6, and 9, respectively. YDs of the treatment sample with 0.6 % aurone were 0.41, 1.53, and 22.85 % on day 3, 6, and 9, respectively. YDs of the treatment sample with 1.0 % aurone were 0, 0.21, and 0.48 % on day 3, 6, and 9, respectively. These results indicated that 0.6 and 1.0 % aurone could delay the yellowing for at least 3 and 6 d, respectively. The effect of storage time on YDs was significant. The effects of aurone treatments on YDs on day 6 and 9 were significant, respectively. However, the evaluation methods of YD were not completely the same in different studies. Judging YD directly from the pictures of FCWCs during storage might be intuitive. Our pictures showed that 1.0 % aurone delayed the yellowing for at least 6 d. Chitosan (Pen & Jiang, 2003), salicylic acid (Peng & Jiang, 2006), citric acid (Jiang et al., 2004), H_2O_2 (Peng et al., 2008), L-Cys (Li et al., 2017), eugenol (Teng et al., 2020; Zhu et al., 2022), high-pressure CO_2 (Kong et al., 2021), and vit E (Ye et al., 2022) only delayed the browning for at most 3 d. Ascorbic acid could not inhibit the yellowing (Song et al., 2019a). Ferulic acid and hydrogen-rich water only delayed the yellowing for at most 3 d (Li et al., 2022; Song et al., 2019a).

Eriodictyol and naringenin concentrations

The results of eriodictyol and naringenin concentrations of the controls and the treatment samples with 0.2, 0.6, and 1.0 % aurone were list in Fig. 2B and 2C, respectively.

Eriodictyol concentrations of the controls were 4.5 and 15.4 × 10^{-4} % on day 6 and 9, respectively. It could not be detected on day 0 and 3. Eriodictyol concentrations of the treatment sample with 0.2 % aurone were 4.4 and 15.2 × 10^{-4} % on day 6 and 9 respectively, while it could not be detected on day 0 and 3. Eriodictyol concentrations of the treatment sample with 0.6 % aurone were 4.4 × 10^{-4} % on day 9, while it could not be detected on day 0, 3 and 6. Eriodictyol concentrations of the treatment sample with 1.0 % aurone could not be detected during

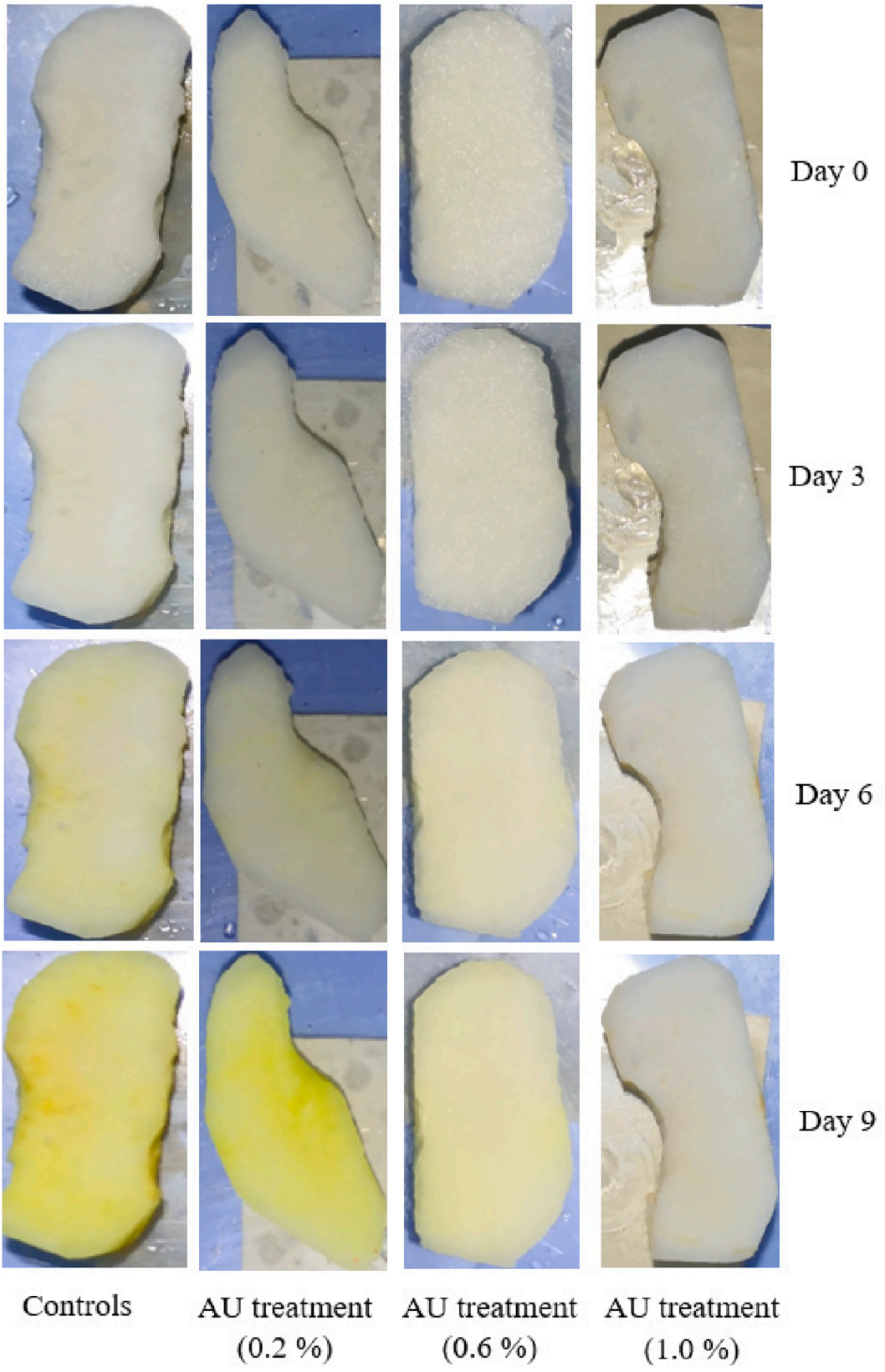

Fig. 1. Photographs of the controls and the treatments samples with 0.2, 0.6 and.

storage. The variation trend of eriodictyol concentration was in accord with that of YD. It suggested that eriodictyol was the main yellowing product and aurone reduced eriodictyol concentration. The standard substance of eriodictyol was yellow powder. It further indicated that eriodictyol led to the yellowing of FCWCs. The effect of storage time on eriodictyol concentration was significant except the treatment with 1.0 % aurone. The effects of aurone treatment on eriodictyol concentration on day 6 and 9 were significant, respectively. In the Song's and Kong's

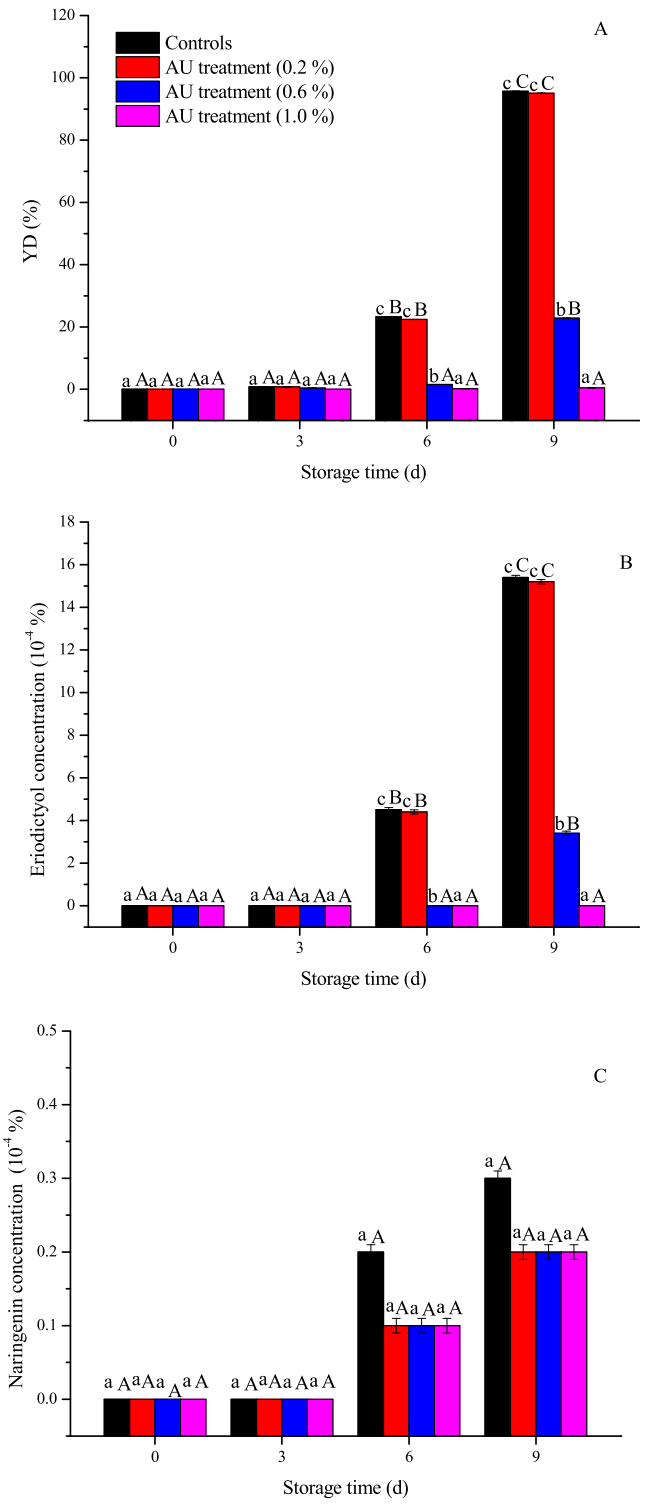

Fig. 2. (A) YD, (B) eriodictyol concentration, and (C) naringenin concentration of the controls and the treatment samples with 0.2 %, 0.6 %, and 1.0 % AU, respectively. Data were expressed as means ± SD (6 repetitions, each replicate used 3 crispers). Different lowercase letters indicated the significant difference by Tukey test ($P < 0.05$) between different treatments on the same day. Different capital letters suggested the significant difference by Tukey test ($P < 0.05$) between different storage times for the same treatment.

studies, eriodictyol concentration of FCWCs could not be detected on day 0 and 3, and increased slowly after day 3 (Kong et al., 2021; Song et al., 2019a). In different studies, eriodictyol concentrations might be not exactly the same because the measurement methods and the WCs species were not exactly the same. The variation trend of eriodictyol concentration of FCWCs in our study was in accord with that in Song,s and Kong,s studies.

Naringenin concentrations of the controls were 0.2 and 0.3 × 10^{-4} % on day 6 and 9, respectively. It could not be detected on day 0 and 3. Naringenin concentrations of the treatment sample with 0.2 % aurone were 0.1 and 0.2 × 10^{-4} % on day 6 and 9 respectively, while it could not be detected on day 0 and 3. Naringenin concentrations of the treatment sample with 0.6 % aurone were 0.1 and 0.2 × 10^{-4} % on day 6 and 9 respectively, while it could not be detected on day 0 and 3. Naringenin concentrations of the treatment sample with 1.0 % aurone were 0.1 and 0.2 × 10^{-4} % on day 6 and 9 respectively, while it could not be detected on day 0 and 3. For the treatment sample with 0.6 % aurone and the treatment sample with 1.0 % aurone, the variation trends of naringenin concentration were not in accord with that of YD. It suggested that naringenin might be not the yellowing product and aurone could not reduce naringenin concentration. The standard substance of naringenin was white acicular crystalline powder. It further indicated that naringenin did not lead to the yellowing of FCWCs. The effect of storage time on naringenin concentration was not significant. The effect of aurone treatment on naringenin concentration was not significant. In the Song's and Kong's studies, naringenin concentrations of FCWCs could not be detected on day 0 and 3, and increased slowly after day 3 (Kong et al., 2021; Song et al., 2019a). The variation trend of naringenin concentration of FCWCs was in accord with that of YD in Song's and Kong's studies. Our findings were different from the results of Song's and Kong's studies. In different studies, different measuring methods and different WCs varieties might affect naringenin concentration.

Enzyme activities

The results of PPO, POD, and PAL activities of the controls and the treatment samples with 0.2, 0.6, and 1.0 % aurone were list in Fig. 3, respectively.

PPO activities of the controls and the treatment samples with 0.2, 0.6, and 1.0 % aurone increased slightly during storage and were between 15 and 17 U s^{-1} kg^{-1}. The effect of storage time on PPO activity was not significant. The effect of aurone treatment on PPO activity was not significant. PPO activity was very low. Moreover, the mixed solution used to measure PPO activity did not yellow when we measured PPO activity. These suggested that the yellowing of FCWCs was different from enzymatic browning of fresh-cut apple (Zha et al., 2022; Zhu et al., 2022), potato (Liu et al., 2022; Qiao et al., 2022), broccoli (Guo et al., 2022; Yang et al., 2022), and lettuce (Liang et al., 2022) et al.. PPO could not catalyze the yellowing of FCWCs. Aurone could not inhibit PPO activity. PPO activity was very high in fresh-cut apple (Zha et al., 2022; Zhu et al., 2022), potato (Wang et al., 2022; Xu et al., 2022), broccoli (Guo et al., 2022; Yang et al., 2022) and lettuce (Liang et al., 2022), and increased rapidly during storage. Enzymatic browning was mainly catalyzed by PPO. In Jiang's, Kong's, Pen's, and Teng's studies, they thought PPO catalyzed the browning of FCWCs (Jiang et al., 2004; Kong et al., 2021; Pen & Jiang, 2003; Peng et al., 2008; Peng & Jiang, 2006; Teng et al., 2020; You et al., 2012). In Li's and Song's studies, they thought PPO did not catalyze the yellowing of FCWCs (Li et al., 2022; Song et al., 2019a). The result of our study was in accord with that of Song and Li. In different studies, different measuring methods and different WCs varieties might affect PPO activity.

POD activities of the controls were 22253.5, 22550.1, 25500.3, and 27667.7 U s^{-1} kg^{-1} on day 0, 3, 6, and 9, respectively. POD activities of the treatment sample with 0.2 % aurone were 22245.8, 22481.1, 25405.5, and 27649.5 U s^{-1} kg^{-1} on day 0, 3, 6, and 9, respectively. POD activities of the treatment sample with 0.6 % aurone were 22240.5,

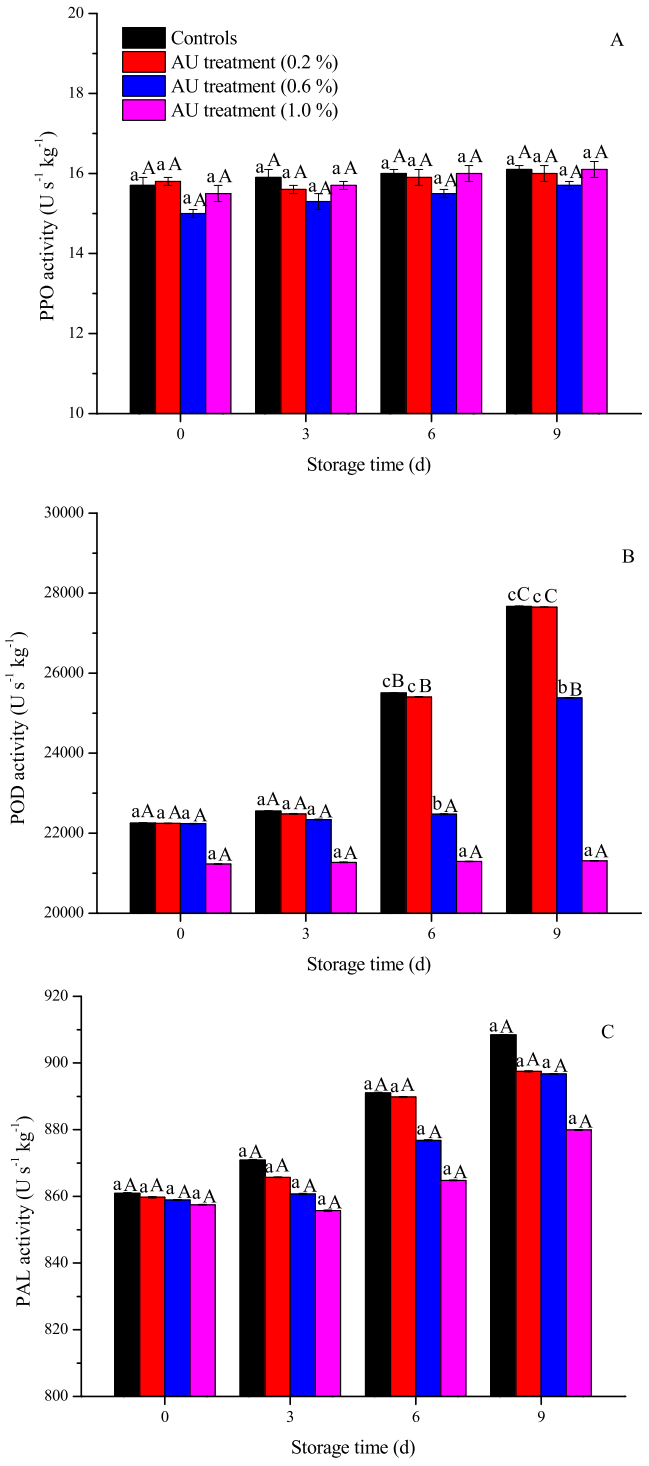

Fig. 3. (A) PPO activity, (B) POD activity, and (C) PAL activity of the controls and the treatments samples with 0.2, 0.6 and 1.0 % AU. Different capital letters indicated the significant difference by Tukey test ($P < 0.05$) between different storage times for the same treatment. Different lowercase letters suggested the significant difference by Tukey test ($P < 0.05$) between different treatments on the same day. Data were expressed as means ± SD (6 repetitions, each replicate used 3 crispers).

22341.5, 22473.6, and 25381.7 U s^{-1} kg^{-1} on day 0, 3, 6, and 9, respectively. POD activities of the treatment sample with 1.0 % aurone were 21230.1, 21271.5, 21293.2, and 21311.4 U s^{-1} kg^{-1} on day 0, 3, 6, and 9, respectively. The variation trend of POD activity was in accord with that of YD. The effect of storage time on POD activity was significant except the treatment with 1.0 % aurone. The effects of aurone treatment on POD activity on day 6 and 9 were significant, respectively. POD activity was very high. Moreover, the mixed solution used to measure POD activity yellowed rapidly when we measured POD activity. These suggested that POD catalyzed the yellowing of FCWCs and aurone inhibited POD activity. In the studies of chitosan, salicylic acid, citric acid, H_2O_2, short-term anoxia, eugenol, and high-pressure CO_2 inhibiting the browning of FCWCs, these researchers thought POD catalyzed the browning of FCWCs and its role is secondary (Jiang et al., 2004; Kong et al., 2021; Pen & Jiang, 2003; Peng et al., 2008; Peng & Jiang, 2006; Teng et al., 2020; You et al., 2012; Zhu et al., 2022). In the studies of ferulic acid and hydrogen-rich water inhibiting the yellowing of FCWCs, researchers thought POD catalyzed the yellowing of FCWCs and its role is secondary (Li et al., 2022; Song et al., 2019a). We found that POD catalyzed the yellowing of FCWCs and its role is primary. In different studies, different measuring methods and different WCs varieties might affect POD activity.

PAL activities of the controls and the treatment samples with 0.2, 0.6, and 1.0 % aurone increased slightly during storage and were between 850 and 910 U s^{-1} kg^{-1}. The effect of storage time on PAL activity was not significant. The effect of aurone treatment on PAL activity was not significant. Moreover, the mixed solution used to measure PAL activity did not yellow when we measured PAL activity. It suggested that PAL did not catalyze the yellowing of FCWCs and aurone did not inhibit PAL activity. In the studies of chitosan, salicylic acid, citric acid, H_2O_2, short-term anoxia, eugenol, and high-pressure CO_2 inhibiting the browning of FCWCs, these researchers thought PAL catalyzed the browning of FCWCs and its role is secondary (Jiang et al., 2004; Kong et al., 2021; Pen & Jiang, 2003; Peng et al., 2008; Peng & Jiang, 2006; Teng et al., 2020; You et al., 2012). In the studies of ferulic acid and hydrogen-rich water inhibiting the yellowing of FCWCs, researchers thought PAL catalyzed the yellowing of FCWCs and its role is primary (Li et al., 2022; Song et al., 2019a). Our findings were different from theirs. In different studies, different measuring methods and different WCs varieties might affect PAL activity.

Microbial colonies

The results of microbial colonies were illustrated in Fig. 4. Number of thermophilic bacteria colonies and number of yeasts and molds colonies of the controls and the treatment samples with 0.2, 0.6, and 1.0 % aurone increased gradually during storage and did not exceed the safe value. The effect of storage time on number of microbial colonies was not significant. The effect of aurone treatment on number of microbial colonies was not significant.

Suppression type of aurone

The Lineweaver-Burk plot, the Slope-Inhibitor concentration plot, and the Intercept-Inhibitor concentration plot of POD was expounded in Fig. 5.

The Lineweaver-Burk plot of POD suggested that the inhibition of aurone to POD was a kind of mixed inhibition. A mixed inhibition is the combination of a competitive inhibition and an anticompetitive inhibition (Yu et al., 2021). The Slope-Inhibitor concentration plot and the Intercept-Inhibitor concentration plot were all a straight. These further verified that the inhibition of aurone to POD was a kind of mixed inhibition. Consequently, aurone was bound to the active center of POD and the POD - substrate complex simultaneously. The K_i and K_{is} values of aurone were 0.2817595 and 0.28175909 %, respectively. These revealed that SE directly bound to the free POD was more than that of the POD- substrate complex, but not significantly.

Conclusions

In conclusion, the study showed that the color change of FCWCs was

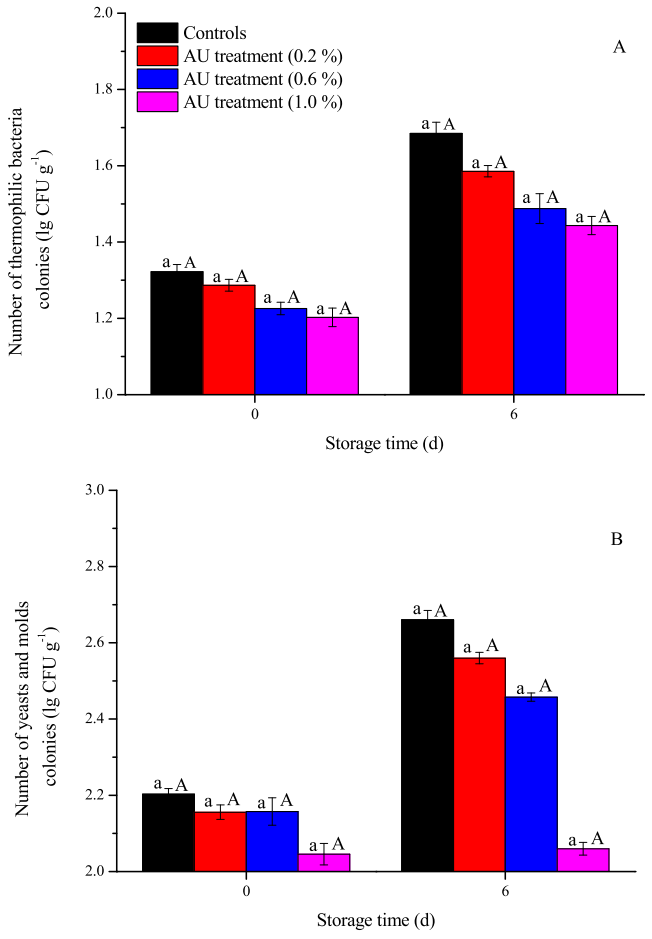

Fig. 4. (A) number of thermophilic bacteria colonies and (B) number of yeasts and molds colonies of the controls and the treatments samples with 0.2, 0.6, and 1.0 % AU. Different capital letters indicated the significant difference by Tukey test ($P < 0.05$) between different storage times for the same treatment. Different lowercase letters suggested the significant difference by Tukey test ($P < 0.05$) between different treatments on the same day. Data were expressed as means ± SD (6 repetitions, each replicate used 3 crispers).

the yellowing, which was catalyzed by POD. This study showed that 0.6 and 1.0 % aurone could delay the yellowing of FCWCs effectively. Aurone decreased the yield of eriodictyol and inhibited POD activity. AU had no significant effect on naringenin concentration, PPO activity, PAL activity, number of thermophilic bacteria colonies, number of yeasts and molds colonies of FCWCs. Aurone inhibited POD activity by competing for the active center of POD and the POD- substrate complex. It has not been studied that how aurone reduced the yield of eriodictyol. In the future, we will use a highly performance liquid chromatography - mass spectrometry (HPLC - MS) to isolate and identify the yellowing substrates to determine how eriodictyol was generated and how aurone reduced the yield of eriodictyol. As a new, convenient, safe, and effective postharvest technology, aurone treatment cloud be used to inhibit the yellowing of FCWCs.

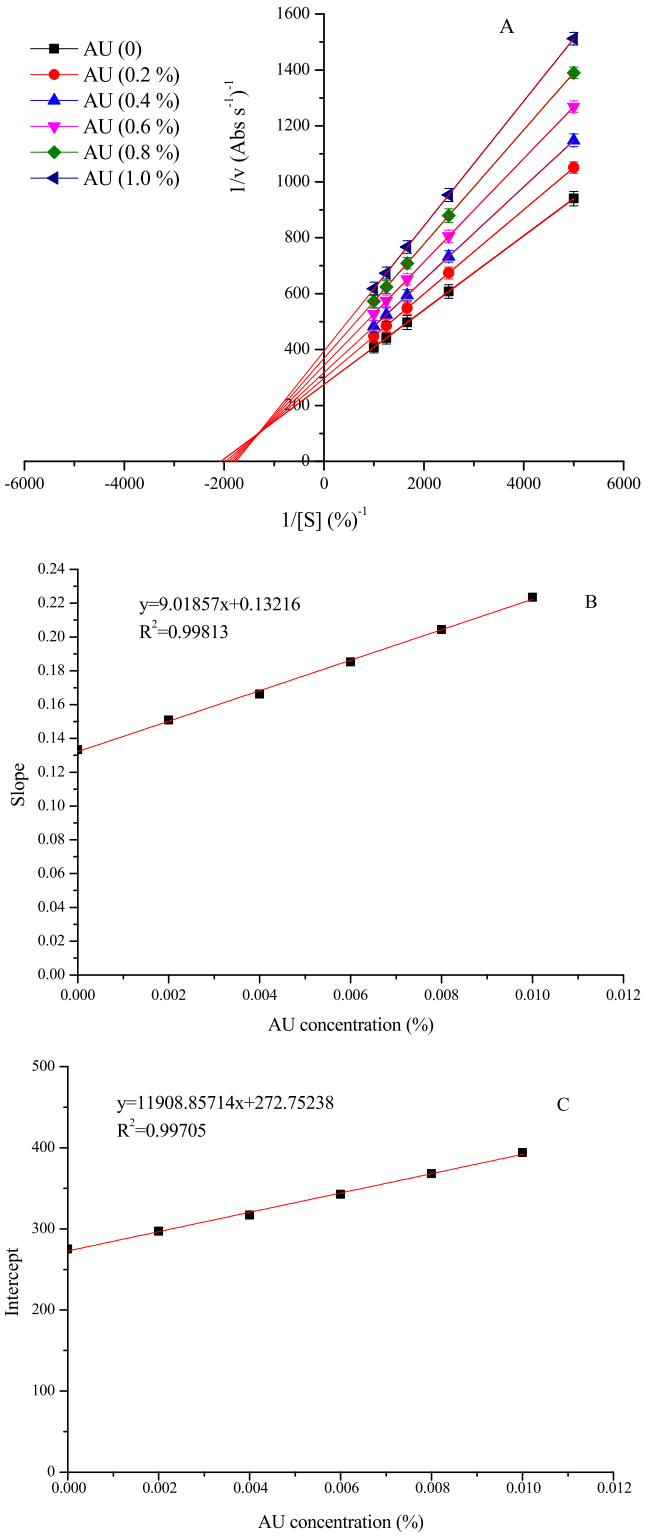

Fig. 5. (A)The Lineweaver-Burk plot, (B) the Slope-Inhibitor concentration plot, and (C) the Intercept-Inhibitor concentration plot of POD.

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CRediT authorship contribution statement

Ao Zhang: Formal analysis. **Liru Mu:** Formal analysis. **Yunmin Shi:** Formal analysis. **Yang Liu:** Formal analysis. **Yan Deng:** Formal analysis. **Yu Lao:** Formal analysis. **Wangping Liu:** Formal analysis. **Shiyun Wang:** . **Yulin Li:** Funding acquisition, Supervision, Conceptualization, Methodology, Validation, Resources, Data curation, Visualization, Investigation, Writing – original draft, Writing – review & editing, Supervision. **Jianjun Hou:** Funding acquisition. **Xian Xia:** Project administration.

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.

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Further reading

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