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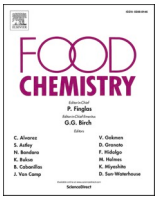

Effects of electrolysed water and levulinic acid combination on microbial safety and polysaccharide nanostructure of organic strawberry

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ABSTRACT

This study aimed to better understand the effects of acidic electrolysed water (AEW, 4 mg/L) and levulinic acid (LA, food grade, 2%) combination on organic strawberry over 7 days. This combined method reduced the population of strawberry's natural microbiota by 1–2 log CFU/g and kept the level of inoculated *Escherichia coli* O157:H7 and *Salmonella* below the detection limit (2 log CFU/g) during the whole storage period. Meanwhile, AEW + LA did not affect the physicochemical qualities of strawberries significantly, maintaining most texture and biochemical attributes at an acceptable level (e.g., firmness, colour, soluble solids content and organic acid content). Atomic force microscopy further revealed that the treatment containing LA preserved the sodium carbonate soluble pectin (SSP) nanostructure best by maintaining their length and height, and slowed the breakdown of SSP chains by promoting acid-induced bonding and soluble pectin precipitation. These results demonstrated that low concentration AEW and LA combination is a promising sanitising approach for organic strawberry preservation.

1. Introduction

Strawberry with its sweet and sour taste and nutrition value is very popular in the market that its sales rose from 1.4 to 2.3 billion in the past decade (Perez & Plattner, 2013). However, some foodborne outbreaks associated with fresh strawberries have been reported in recent years. One well-known and serious outbreak of *Escherichia coli* O157:H7 related to the consumption of contaminated strawberries was reported within Oregon in 2011, involving 15 cases and 2 deaths (Laidler et al., 2013). Another case of *Salmonella* infection was surveilled by Food and Drug Administration (FDA), reporting 1 in 143 lots of imported strawberry into the U.S. was contaminated by *Salmonella* (FDA, 2001). Although most foodborne outbreaks came from conventional strawberries, the use of animal manures as natural fertilisers might result in even higher contamination level of microorganisms in strawberries from organic systems than those from conventional farms, making sterilisation process more important to ensure organic strawberries' safety.

Acidic electrolysed water (AEW) has gained interest recently as an advanced and novel sanitiser in food industry due to its competitive advantages over conventional chemical reagents, such as being non-corrosive, safe, environmentally friendly and economical since the resources of AEW are just salt (sodium chloride), electricity and water (He et al., 2022; Zhao, Li, & Yang, 2021). The major antimicrobic component generated from electrolysis is HClO, which is electrically neutral and can pass through microbial cell membrane to induce intracellular pH imbalance, leading to cell death. However, considering the strict regulations regarding chlorine residue in organic food industry, the residual chlorine concentration in AEW contacted with organic food must be kept as low as that permitted to exist in the disinfected drinking water, i.e., not be over 4 mg/L (NOP 5026, 2011). So, to get a desirable sanitising result, low concentration AEW has to be used together with another method or reagent for organic food application.

Most of organic acids are generally recognised as safe (GRAS) and exhibit strong bactericidal effects on various pathogens (Chen et al., 2022). Multiple studies showed the synergistic antimicrobial effect of electrolysed water combined with different organic acids, such as citric acid, fumaric acid, lactic acid, on a variety of fresh produce (Tango et al., 2017). However, levulinic acid (LA), another organic acid also designated as GRAS by the U.S. Food and Drug Administration (FDA), has not received as much attention as its counterparts. In most of existing research works, LA was usually combined with sodium dodecyl sulfate (SDS) as an antimicrobial intervention for food preservation. For

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example, more than 2 log colony forming unit (CFU) reduction of *S.* Typhimurium on apples and celery was observed after treatment with 0.5% LA + 0.05% SDS (Zhou, Doyle, & Chen, 2020). Up to now, limited attention was paid to the sanitising effect of LA on microbes during the preservation of organic produce.

Therefore, the main objectives of this study were to use low concentration AEW and LA combination to enhance microbial safety of strawberries and keep the quality properties stable during storage, expecting to prolong the shelf life. Moreover, considering the softening of strawberries is strongly associated with the assembly and dynamics of pectin, the changes of pectin structure and content were particularly studied. The results of this project would contribute to develop a potential organic-compatible sanitising method and provide an improved understanding on the changes of nutrient quality, texture, and pectin characteristics of strawberry during storage.

2. Materials and methods

2.1. Fruit materials and sanitising treatments

Organic strawberries (*Fragaria* × *annanassa* Duch.) imported from South Korea were obtained from a local produce market and transferred to laboratory immediately, which were stored in fridge before being treated within 24 h. The fruit that in medium size, uniform colour and mature stage were selected, then be divided into four treatment groups randomly: 1) deionised water (DW as control), 2) acidic electrolysed water (AEW, free available chlorine (FAC): 4 mg/L, achieved through 0.9% sodium chloride solution being electrolysed by an electrolysis device (ROX-10WB3, Hoshizaki Electric Company, Japan)), 3) levulinic acid (LA, 2% v/v generated by a food-grade solution of 97% v/v, Sigma-Aldrich, USA), and 4) the LA and AEW combination. The characteristics of all kinds of sanitising solution are presented in Table S1. The FAC was determined by a chlorine test kit (Merck, Darmstadt, Germany), pH was measured with a pH meter (Thermo Orion pH meter, Waltham, USA), and the ORP value was detected with an ORP meter (HM Digital ORP-200, Culver City, USA). Strawberries from each group were immersed in a big beaker containing 2 L of the corresponding treatment solution for 5 min. The beakers were covered by aluminium foil to avoid volatilisation of chlorine and the FAC value of AEW-included solution can be maintained at 2–4 mg/L during treatments (Zhao et al., 2022). After air-drying under room temperature (25 °C) for 30 min, strawberries were randomly divided and packed in zipper bags (16.8 cm × 8.2 cm) with some holes before putting in a 4 °C refrigerator for a week storage. Aside from strawberries on day 0, those from each treatment group on each sampling day (day 0, 1, 3, 5, 7) were removed from the refrigerator and placed under room temperature (25 °C) for 2 h before analysing the following physicochemical properties.

2.2. Bacterial inoculation on strawberry

Escherichia coli O157:H7 ATCC 43895 and *Salmonella* Typhimurium ATCC 14028 were used as inoculation strains on the surfaces of strawberries in this study. After collected from ATCC and grown until stationary phase in tryptic soy broth, they were diluted to the concentration of 10^7 CFU/mL by using 0.1% peptone water (PW). Twenty strawberries for each treatment group (10 for each bacterium inoculation, 2 for each sampling day), i.e., a total of 80 strawberries were immersed in *E. coli* or *S.* Typhimurium suspensions for 5 min and then underwent 30 min of air-drying inside a laminar flow biosafety cabinet. After strawberries being inoculated, sanitising treatments as mentioned in Section 2.1 were performed.

2.3. Microbiological analysis

Two strawberries (around 25 g) from each group on each sampling day were homogenised with 225 mL 0.1% sterile PW within a stomacher bag with the use of a stomacher (Masticator Stomacher, IUL Instruments, Germany). Plate spreading was conducted with 0.1 mL of the diluted solution in PW and various dilutions were assessed. The enumeration of *E. coli* was performed on Sorbitol-MacConkey agar (SMAC, Oxoid, UK), while that of *S.* Typhimurium was done on xylose lysine deoxycholate agar plates (XLD, Becton Dickinson), respectively, by counting the grown colonies after 24 h in a 37 °C incubator. Besides, for strawberries' own bacteria, aerobic mesophilic count (AMC) and aerobic psychrotrophic count (APC) were obtained from plate count agars (PCA, Oxoid, Britain), which underwent incubation for 48 h at 37 °C and for 10 days at 7 °C, respectively. Yeasts and moulds enumeration was carried out with potato dextrose agars (PDA, Oxoid, Britain), which were incubated for 72 h at 25 °C. All the results were exhibited as 'log CFU/g sample'.

2.4. Quality analysis

2.4.1. Weight loss and firmness

Twenty strawberries from each group at each sampling day were included in the weight loss study. After weighing each strawberry, weight loss (%) calculation was conducted with the following equation: weight loss (%) $=(W_0 - W_x)/W_0$, where W_0 means the original weight and W_x represents the current weight. This process was repeated 3 times.

Fifteen strawberries in each treatment group were used to assess firmness. Each of them from each sampling day was divided into two 2 cm × 2 cm × 1 cm cubes for compression. TA-XT2i texture analyser (Stable Micro Systems Ltd., Godalming, Surrey, UK) along with a P35 probe (6 mm) was used after calibration. The speeds of pre-test, test, and post-test were set as 3, 1, and 5 mm/s, respectively, and the trigger force was set as 0.1 N. The results were expressed by the maximum force encountered.

2.4.2. Soluble solids content and titratable acidity

Ten strawberries from each sanitising treatment group were used to analyse the soluble solids content (SSC) with a digital refractometer (Atago Co. Ltd, Tokyo, Japan). After grinding each strawberry and centrifugating (4000 × *g*, 10 min, 20 °C), the supernatant was measured, and the results were displayed as °Brix.

The titratable acidity (TA) of strawberries in each treatment group during storage was determined by titrating 20 mL diluted juice (10 g strawberries grinded in 100 mL distilled water) with standard 0.1 M NaOH. The results were described in malic acid equivalents after titration was terminated when the pH reached 8.2.

2.4.3. Colour

Colour information of 15 strawberries from each group at each sampling day was performed with the use of a Minolta Colorimeter (Konica Minolta, Inc., Tokyo, Japan). Five colorimetric characters (L*, a*, b*, c*, h°) were measured for each strawberry on two different surface locations after calibration. According to the International Commission on Illumination (CIE) colour system, L*, a* and b* are three coordinates in colour space, in which L* reflects lightness, a* indicates green–red and b* is the blue-yellow coordinate, respectively. The chroma (c*) and hue angle (h°) values were calculated using Eq. (1) and Eq. (2), respectively:

$$c* = \sqrt{a*^2 + b*^2} \quad (1)$$

$$h^\circ = \tan^{-1}\left(\frac{b*}{a*}\right) \quad (2)$$

In total, 30 replicates were tested for each group on each sampling day, and each measure was run with three replications.

2.5. Biochemical analysis

2.5.1. Total phenolic content

A spectrophotometer (Shimadzu, Corporation, Kyoto, Japan) was used for analysing total phenolic content (TPC) by setting the absorbance at 760 nm, according to the method explained by Liu, Tan, Yang and Wang (2017) with slight adjustments. Standard gallic acid in gradient concentrations (0, 0.01, 0.05, 0.1, 0.25, 0.5 mg/mL) was prepared in methanol as reference. Two grams of strawberries from each group were grinded with 20 mL 1% HCl-methanol. Then 100 μL extracts were taken with the addition of 100 μL Folin-Ciocalteu reagent and 1 mL 7% sodium carbonate before kept in dark for 90 min. The result was indicated as 'mg gallic acid equivalent (GAE) per gram of fresh weight (FW)'.

2.5.2. Ascorbic acid content

For the ascorbic acid content (AAC), above spectrophotometer was also used for absorbance measurement at 534 nm. Ten grams of strawberries from each group underwent ice grinding with 100 mL 50 g/L trichloroacetic acid (TCA) solution. Then 1 mL sample was mixed with 1 mL 50 g/L TCA and 1 mL 100% ethanol after centrifugation (4000 × *g*, 10 min, 4 °C). After mixing thoroughly, additional 0.5 mL 0.4%

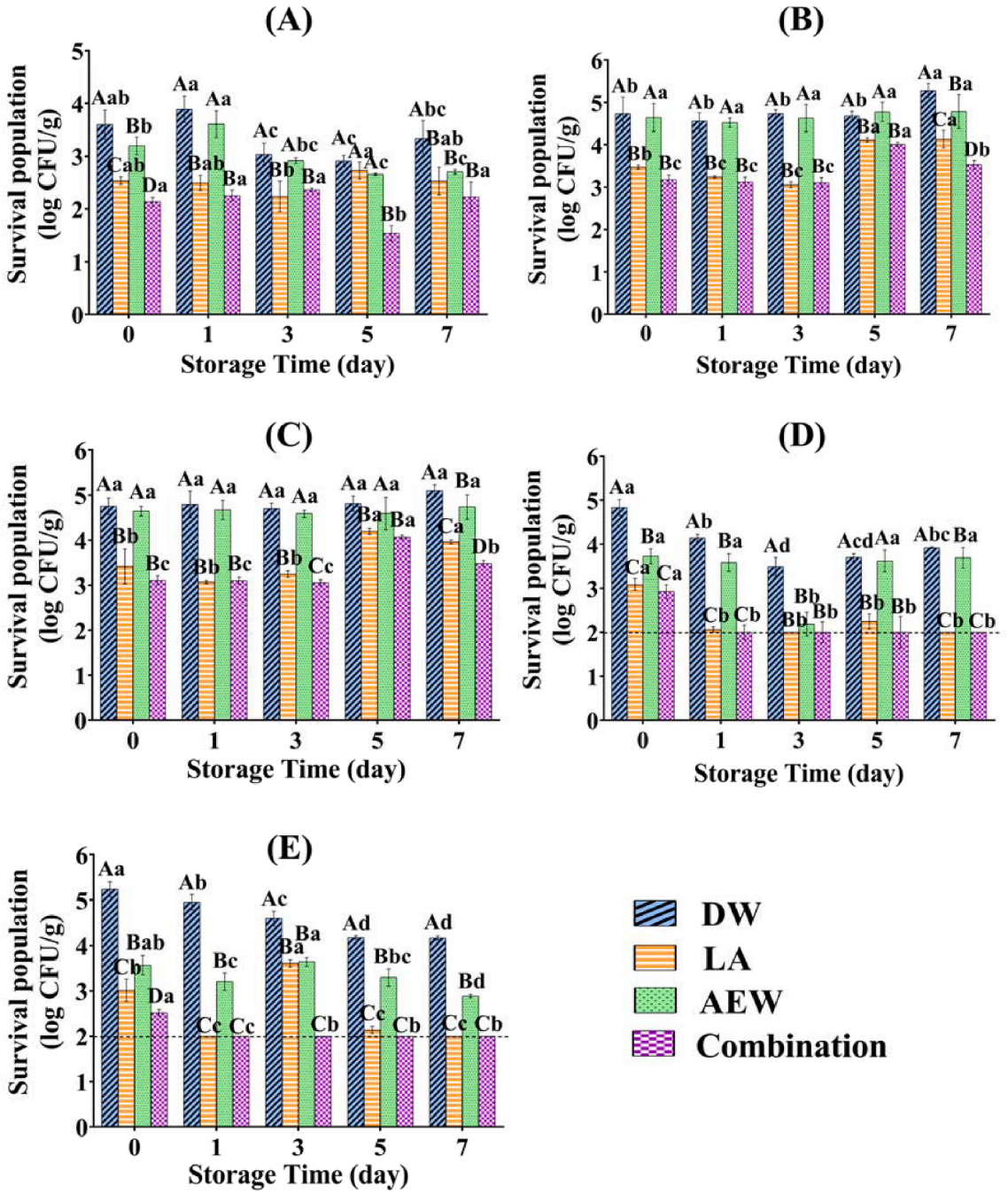

Fig. 1. Effect of different sanitising treatments to strawberries on aerobic mesophilic count (A), aerobic psychrotrophic count (B), yeasts and moulds (C), *E. coli* O157:H7 ATCC 43895 (D) and *Salmonella* Typhimurium ATCC 14028 (E) during storage period. Data are displayed as mean values ± standard deviation. Within the same storage period under different treatments, significant differences are shown by different capital letters; for the same treatments at different storage times, significant differences are shown by different lowercase letters ($P < 0.05$). DW: deionised water; LA: levulinic acid; AEW: acidic electrolysed water; Combination: AEW + LA.

phospho-ethanol, 1.0 mL 5 g/L 4,7-diphenyl-1,10-phenanthroline-ethanol (BP-ethanol), and 0.5 mL 0.3 g/L $FeCl_3$-ethanol was added, and the mixture stayed for 60 min at 30 °C before absorbance analysis.

2.5.3. Organic acid content

Organic acid profile of strawberries was investigated with the help of high performance liquid chromatography (HPLC). Five grams of strawberries were firstly grinded with 25 mL 10% ethanol and transferred for ultrasonic mixing for 30 min. After adding 1 mL 0.5 mol/L sulfuric acid, supernatant was collected through centrifugation (4000 × *g*, 10 min, 4 °C) and subsequently filtered through 0.45 μm membrane before HPLC analysis.

The mobile phase used in this study was A: 0.005 M sulfuric acid and B: methanol in the proportion of 98% and 2%. The flow rate was set as 0.5 mL/min along with the column temperature of 30 °C and the sample temperature of 4 °C. Then 20 μL of the solution was injected for 30-min running per sample and the final tested wavelength was set between 200 and 300 nm. The column was ZORBAX Eclipse XDB-C18 Separation column (4.6 × 250 mm, 5 μm; Agilent Technologies, Inc., Richardson, TX, USA), and the detector was PDA 2996 detector (Waters, Milford, MA, USA).

2.6. Pectin extraction and content analysis

The extraction methods of cell wall material (CWM), water-soluble pectin (WSP), chelate-soluble pectin (CSP), and sodium carbonate-soluble pectin (SSP) referred to paper from Chen et al. (2011) with slight adjustments. Fifteen grams of strawberries from each treatment group were placed in the boiling water bath for 20 min after the addition of 200 mL 80% ethanol. After centrifugation (8000 × *g*, 15 min, 4 °C), the solid residue was collected and immersed in 30 mL DMSO/H_2O (9:1) at 4 °C for 12 h. Then the residue was mixed with 200 mL chloroform/ethanol (2:1) for 10 min after filtration. CWM was extracted when the residue was washed with 200 mL acetone until colour turned white. For different portions of pectin, CWM residue was further dipped in ultra-purified water for 4-h shaking at 25 °C, then it went through centrifugation (10000 × *g*, 10 min, 4 °C) for supernatant collection. This process repeated twice more and the WSP was collected in triplicate supernatants. Remaining pellet was further immersed in 10 mL of 50 mM cyclohexane-*trans*-1,2-diamine tetra-acetate (CDTA) at 25 °C for 4 h and then centrifugated (10000 × *g*, 10 min, 4 °C) to fractionate supernatant, which was also repeated twice more as above. The combination of three supernatants was regarded as CSP. Final remnant was subsequently immersed in 10 mL Na_2CO_3/CDTA (50 mmol/L/2 mmol/L) for 4 h at 25 °C three times to obtain the supernatants which were known as SSP.

The quantitative change of each fraction of pectin was analysed by using the method of carbazole colorimetry explained by Chen et al. (2011) with some modifications. One mL of WSP/CSP/SSP extracted solution was mixed well with 6 mL sulfuric acid (98% w/w, Sigma-Aldrich, USA) and located in a boiling water bath for 20 min. Then 0.2 mL of 1.5 g/L carbazole-ethanol solution was added after the solution was cooled to room temperature, followed by a 30-min dark incubation. The absorbance at 530 nm was measured with a spectrophotometer. Pure galacturonic acid (Sigma-Aldrich, USA) in various concentrations acted as a standard. The results were revealed as 'mg per 100 g FW'.

2.7. AFM analysis of pectin morphological changes

Atomic force microscopy (AFM) has been considered as an effective tool to examine the pectin morphological changes for a better understanding of the produce's quality changes. Considering previous studies had found that the degradation of SSP during storage was a major mediator for the softening of strawberry (Chen et al., 2011), the AFM analysis of SSP was conducted in this study. A freshly cleaved mica sheet surface was pipetted with 10 μL diluted SSP solution (~10 μg/mL) and then underwent air-drying before being tapped to AFM specimen discs (TT-AFM workshop, Signal Hill, USA) for characterisation. In this study, Sensaprobe TM190-A-15 tip (Applied Nanostructures, Mountain View, USA) was utilised in the mode of 512 pixels/line and 0.5 Hz scan rate. Gwyddion software was used for the processing of generated AFM images (Chen et al., 2022). Height and length of each single chain were measured from vertical and horizontal side, respectively, and the results were expressed as the frequency (Fq) of the number of chains with different heights or lengths.

2.8. Statistical analysis

All experiments were done with three replications. ANOVA ($P < 0.05$) was utilised to compare means with standard deviation, while Duncan's multiple-range test with an IBM SPSS statistical software (version 24; IBM Corp., Armonk, USA) was used to determine the differences among treatment groups. Representative results in AFM imaging and quantitative analysis were obtained through parallel images. In each group, the parallel number of SSP chains was ca. 100 to make quantitative results more representative.

3. Results and discussion

3.1. Sanitising effects on natural microbiota

Fig. 1A-C show the sanitising effect of different treatments on natural microbiota of strawberries. The initial loads of AMC, APC and yeasts and moulds on strawberry before any treatment was around 3.97, 4.76 and 4.80 log CFU/g, respectively (data not shown). The survival populations of all kinds of microorganisms decreased immediately (day 0) after LA and combination treatment, by comparison with the amounts in each control group, whereas AEW alone showed limited antimicrobial effects on strawberries, which was similar to the results on lettuce surfaces in our previous study (Zhao, Zhao, Phey, & Yang, 2019). During 7-day storage, the amount of aerobic psychrotrophic microorganisms and yeasts and moulds in control and AEW group remained basically unchanged, while the AMC in these two groups decreased gradually, indicating that aerobic mesophilic microorganisms might be more sensitive to the storage temperature (4 °C) and the nutrient content. On the other hand, the lowest level of all microbial counts was found in the combined group in most sampling days, with more than 1.11, 1,75 and 1.60 log CFU/g reductions for AMC, APC and yeast and moulds, respectively at the end of storage period, compared to their respective control group (Fig. 1A-C).

According to a report assessing the microbiological levels of fresh produce sold in Singapore, AMC with an overall range from 1.6 to 5.1 log CFU/g was found in 42 fruit samples (i.e., apples, mangos, and oranges) collected from major supermarkets and local markets. However, the situation was more serious in vegetable samples, as up to 9.1 log CFU/g of AMC was observed in bean sprouts, and the highest level of coliforms and yeasts and moulds was found in both bean sprouts and fresh-cut salads, the latter of which also contained the highest mean APC of 4.9 log CFU/g (Seow, Ágoston, Phua, & Yuk, 2012). Our experimental data were consistent with the statistical results of this published report. On the other hand, the Agri-Food and Veterinary Authority (AVA) of Singapore regulates that the aerobic bacterial count on ready-to-eat foods should not exceed 5.0 log CFU/g (Agri-Food and Veterinary Authority of Singapore, 2005). Although the microbial loads on our strawberry samples and on most of Seow et al.'s produce samples purchased from local supermarkets were within this rule, effective control measures should still be implemented, considering increased microbial populations over extended storage time as shown in our control groups might pose a health risk to consumers. Therefore, the effective sanitising results brought by the combination of LA and AEW on strawberries shall also apply to other fresh produce, enhancing the microbiological quality of ready-to-eat fruits and vegetables sold in Singapore.

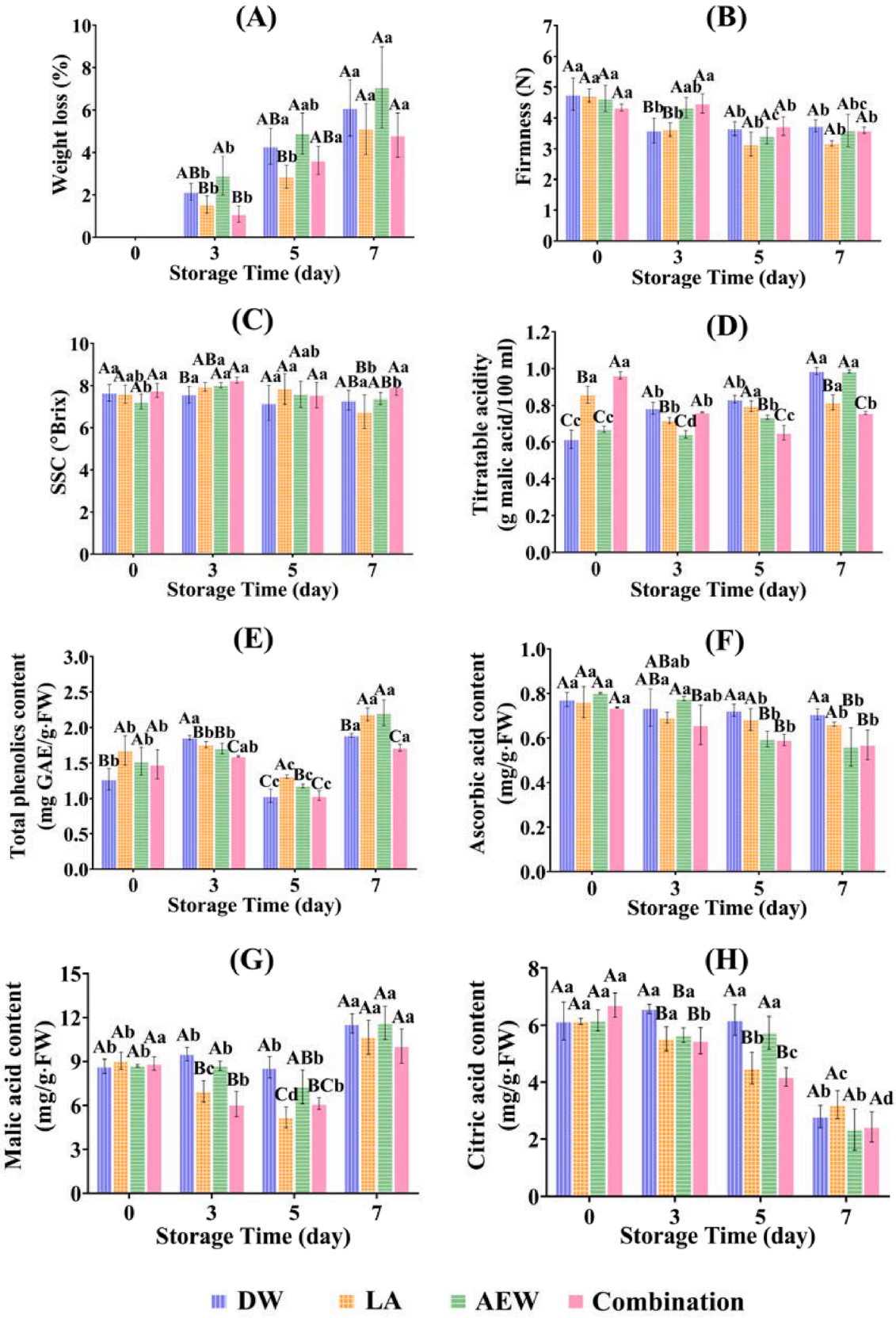

Fig. 2. Effect of different sanitising treatments to strawberries on weight loss (A), firmness (B), soluble solids content (SSC) (C), titratable acidity (TA) (D), total phenolics content (E), ascorbic acid content (F), malic acid content (G), and citric acid content (H) during storage period. Data are displayed as mean values ± standard deviation. Within the same storage period under different treatments, significant differences are shown by different capital letters; for the same treatments at different storage times, significant differences are shown by different lowercase letters ($P < 0.05$). DW: deionised water; LA: levulinic acid; AEW: acidic electrolysed water; Combination: AEW + LA.

3.2. Sanitising effects on E. coli O157:H7 and S. Typhimurium

Survival populations of two inoculated bacteria, *E. coli* O157:H7 and *S*. Typhimurium after each sanitising treatment are shown in Fig. 1D and E. The initial inoculums of *E. coli* O157:H7 and *S*. Typhimurium on strawberries before any treatment were 5.56 and 5.60 log CFU/g, respectively (data not shown). The levels of *E. coli* O157:H7 decreased immediately after all treatments, with 1.74, 1.10 and 1.91 log CFU/g reductions observed in LA, AEW and their combination treated group, respectively, as compared to the control group (Fig. 1D, day 0). Whereas in *S*. Typhimurium, more significant antimicrobial effects were observed, with 2.23, 1.67 and 2.73 log CFU/g reductions observed from the surface of strawberries after 5-min immersion washes in the solution of LA, AEW and their combination, respectively (Fig. 1E, day 0). During storage, the survival patterns in the two bacteria also showed some differences, as the populations of *E. coli* O157:H7 in control and AEW group decreased first and increased gradually after day 3. Opposite trends occurred in *S*. Typhimurium, whose levels in both LA and AEW alone group reached the highest point at day 3 and decreased afterwards (Fig. 1D and E). However, the combined treatment could keep the population of both bacteria below the detection limit (2 log CFU/g) during the whole storage period, which cannot be achieved by other treatment methods, indicating good residue effects of LA and AEW combination against foodborne pathogens.

Although both are Gram-negative strains, *S*. Typhimurium exhibited higher susceptibility to LA and AEW compared to *E. coli* O157:H7, which might be attributed to their different adhesive abilities on strawberry surfaces and different interactions with sanitising agents. de São José, de Medeiros, Bernardes, and de Andrade (2014) found that *E. coli* ATCC 11229 was more hydrophilic than *S*. Enteritidis ATCC 13076, which made *E. coli* adhere better on the surfaces of green pepper and melon. Moreover, other factors such as flagella, fimbriae, and cellular surface proteins could also influence microbial adhesion on the surfaces of fresh produce, adding complexity to subsequent disinfection efficacy (Garrett, Bhakoo, & Zhang, 2008). Although the initial population of both bacteria attached to strawberry surfaces was similar in our study, the probably lower adhesive strength of *Salmonella* might result in its easier inactivation and removal from strawberry surfaces by LA and AEW treatments.

3.3. Quality changes

Although this combination of LA and low concentration AEW was mainly developed as a potential strawberry-compatible disinfecting method, the quality of strawberry during storage after this combined method should better not be compromised simultaneously. Therefore, some physicochemical properties of strawberry during storage were evaluated.

3.3.1. Weight loss and firmness

The weight loss (in %) of strawberry samples treated by different sanitisers after different storage periods is shown in Fig. 2A. As the storage time increased, the weight loss in all groups' samples increased concomitantly, which implied the water vapour pathway in strawberries was active during storage, considering the water transmission and transpiration process is a main reason for weight loss. However, the strawberries treated by the sanitisers containing LA were observed with lower weight loss throughout storage, which might be attributed to the reduced stomatal aperture when the fruit epidermis interacted with LA, leading to the decreased fruit transpiration and respiration rate (Jongsri, Wangsomboondee, Rojsitthisak, & Seraypheap, 2016). Whereas the samples in control and AEW treatment group still had a high respiratory intensity along storage, accompanied by high weight loss.

It has been believed that there is a close correlation between firmness and weight loss in fruits, as firmness character can be highly influenced by the water content. As shown in Fig. 2B, the strawberries in all treatment groups experienced a significant decrease of firmness over 7-day storage, however, no significant differences were shown among the samples from different groups on most sampling days, with even higher firmness shown in samples under AEW-included treatments on day 3. Firmness reduction of fresh produce is usually associated with the degradation of the cell wall, which can be induced by a series of hydrolytic enzymes, such as polygalacturonase, cellulase and β-galactosidase (Bu, Yu, Aisikaer, & Ying, 2013). A previous study found that AEW could help delay the softening process of blueberries by lowering the activity of its cell wall degrading enzymes, thus the degradation of cell wall components like pectin, hemicellulose and cellulose, was retarded (Chen, Hung, Chen, & Lin, 2017). Our results were in agreement with previous findings, indicating that AEW-included sanitising method could be served as a potential postharvest technique to maintain the firmness of strawberries.

3.3.2. Soluble solids content and titratable acidity

SSC is a critical indicator for fruit sweetness and consumer acceptability. Overall, the treatment with single LA, AEW and their combination all did not result in dramatic changes of SSC during storage, and at each sampling point, no significant difference was observed among the samples from different treatment groups generally (Fig. 2C). Considering sugars are the main soluble components in soft fruits, the activity of carbohydrate metabolism in strawberries plays an important role in maintaining SSC. Therefore, the almost constant SSC throughout storage in our study indicated that glycolysis remained in a relatively stable status in LA + AEW treated strawberries, without significant consumption of sugars. However, at the end of storage, the strawberries treated by LA alone showed less SSC compared to those in other groups, which might be attributed to the hydrolysis of sucrose for maintaining respiration, echoing earlier finding of reduced respiration rate in LA-treated strawberries in the end as mentioned above (Shao, Zhang, Niu, & Jiang, 2018).

TA refers to the total acid concentration in food samples and is commonly used to evaluate the fruit taste from sweetness and acidity perspectives. The content of TA was expressed as 'g malic acid per 100 mL' in our study, as malic acid was found to be the most prominent organic acid in our strawberry samples in the Section 3.4.3. The changes of TA in all treated strawberries during a week cool storage are shown in Fig. 2D. A significant increase was seen in the LA and LA + AEW treated samples on the processing day, whereas at the end of storage, opposite trends occurred.

Previous studies revealed that the expression of a series of genes encoding biosynthetic enzymes related to organic acid biosynthetic pathways could be upregulated in plant as a response to external stress, thus the biosynthesis of organic acids, such as the conversion from D-glucose to ascorbic acid, might be stress-inducible and activated under LA stimulation in our strawberry samples, causing higher level of TA at the beginning of storage (Cruz-Rus, Amaya, Sanchez-Sevilla, Botella, & Valpuesta, 2011). On the other hand, the variations in TA for DW and AEW treated strawberries increased gradually during storage, which might be resulted from the active respiration of strawberries in these two groups, accompanied by decreased O_2 and increased CO_2 concentration in the zipper bags, leading to affected enzyme systems and accumulated acid in strawberries. Considering the number of microbial survivals on samples under LA and the combined treatment was the lowest as shown above, less off-flavours and acidic compounds produced by the spoilage microorganisms on fruits could also be a reason explaining the lowest TA level shown in the strawberries from these two groups at the end of storage (Zhao, Ndayambaje, Liu, & Xia, 2020).

3.3.3. Colour

As an important parameter to reflect quality, strawberry skin colour is the most direct influencing factor of consumers' acceptance and buying intention. During storage, colour differences of control and treated strawberries are shown in Table S2. The L* values remained

almost stable in all treated strawberries except for the AEW treated ones with a decreasing trend along storage, indicating that AEW might power the oxidative browning reactions happening on the surfaces of strawberry in the presence of HOCl, thus making the colour become darker. Moreover, the decline in a* and b* values was shown in all treated strawberries throughout storage, indicating that sanitised strawberries appeared less red and less yellow compared to the control counterparts, which might be due to the affected contents of antioxidant compounds and polyphenols in strawberries under LA and AEW treatments (Wang, Cui, Vainstein, Chen, & Ma, 2017).

On the other hand, chroma (c*) and hue angle (h°), which are both calculated from the a* and b* values, represent the quantitative and the qualitative attribute of colour, respectively. In general, a significantly lower c* and h° values were observed in all sanitised samples during storage compared to those from control samples, indicating that the sanitised strawberries became duller in appearance with lower colour intensity. It has been believed that the amount of anthocyanin compounds in fruits is significantly correlated with the colour profiles, and the porphobilinogen biosynthesis from 5-Aminolevulinic acid (ALA) to heme can facilitate anthocyanin accumulation in plant, by stimulating the expression of various genes related to the anthocyanin biosynthesis (Xie et al., 2013). However, LA could lower the activity of ALA dehydrogenase, causing disturbed porphobilinogen biosynthesis and inhibited anthocyanin accumulation (Hara et al., 2019), which might explain why the loss of lightness and fresh red colour occurred in the strawberries treated by sanitisers containing LA in our study.

3.4. Biochemical analysis

3.4.1. Total phenolic content

Phenolic compounds can act as a defence system to prevent fruits from postharvest infection and injuries, as well as exert marked effects on fruit's colour, taste and flavour. Changes in TPC of strawberries under different sanitising treatments are shown in Fig. 2E. Right after the treatments, a significantly higher TPC was observed in all sanitiser-treated samples immediately, with 1.68, 1.52 and 1.48 mg GAE/g shown in samples from LA, AEW, and their combination treatment group, respectively, reaching around 22.8% higher TPC in average than that from DW-treated samples. Similar result was found by Liu, Tan, Yang and Wang (2017), in which higher TPC appeared in hot AEW-treated broccoli immediately than that in control samples, indicating probable accumulation or enhancement of TPC in produce under AEW stress. Throughout storage, TPC in all strawberry samples dropped drastically in the first 5 days while increased again on day 7, with higher number shown in single LA and AEW treated samples most of the time, which might be attributed to their influences on the activation of oxidative enzymes related to the phenolics biosynthesis.

Previous studies found that the change of fruit's taste from bitter to sweet during postharvest storage has certain relations with the reduction of TPC, which is a natural variation during development and ripening of fruit (Parra-Palma, Morales-Quintana, & Ramos, 2020). Therefore, the slight decrease of TPC observed in our samples during early stage of storage might be associated with their gradual aging process. On the other hand, it was reported that tannins can be hydrolysed to produce phenolic compounds in damaged plant cells (Dai et al., 2020), which might explain why the increase of TPC occurred at the later stage of shelf-life in our study.

3.4.2. Ascorbic acid content

Strawberries contain a large amount of ascorbic acid, which is a considerable plus for their popularity among consumers. As shown in Fig. 2F, the initial concentration of ascorbic acid in our strawberry samples reached up to more than 80 mg/100 g, which was relatively high compared to others strawberry cultivars (Urün et al., 2021). Although ascorbic acid is a water-soluble vitamin, no significant differences of AAC was observed between control and sanitised samples on the processing day, indicating its similar solubility in all treatment solutions and no more loss of vitamin C after LA and AEW wash. However, during postharvest storage, AAC of fruits can be affected by various external and internal factors, such as desiccation, oxidation and senescence stress, which can cause a series of metabolic changes associated with ascorbic acid formation. The degradation of ascorbic acid

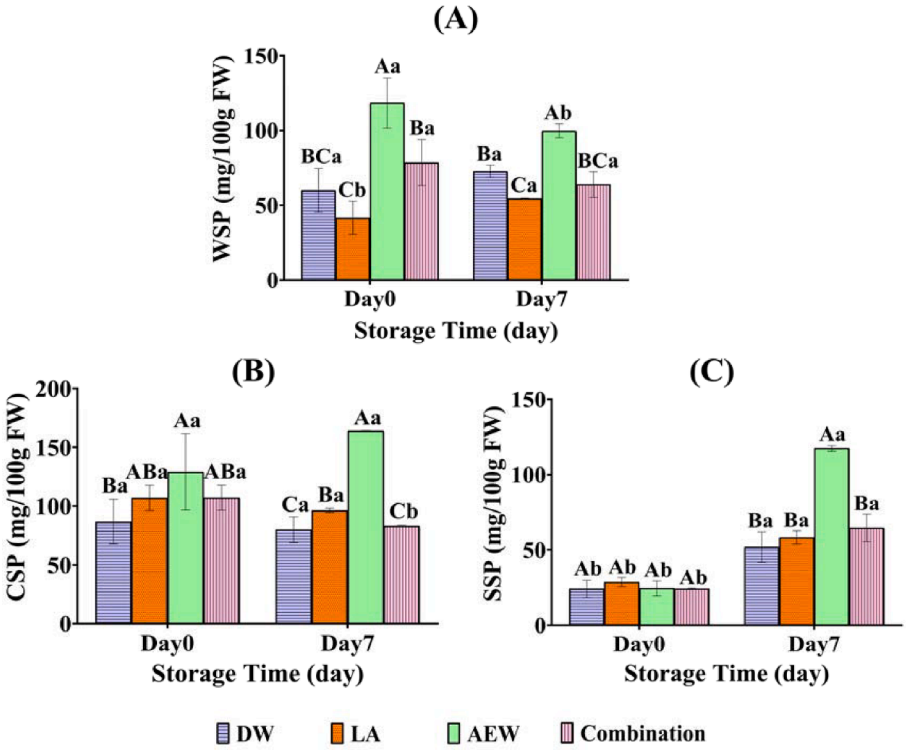

Fig. 3. Effect of different sanitising treatments to strawberries on water soluble pectin (A), chelate soluble pectin (B) and sodium carbonate soluble pectin (C) during storage period. Data are displayed as mean values ± standard deviation. Within the same storage time under different treatments, significant differences are shown by different capital letters; for the same treatments at different storage times, significant differences are shown by different lowercase letters ($P < 0.05$). DW: deionised water; LA: levulinic acid; AEW: acidic electrolysed water; Combination: AEW + LA.

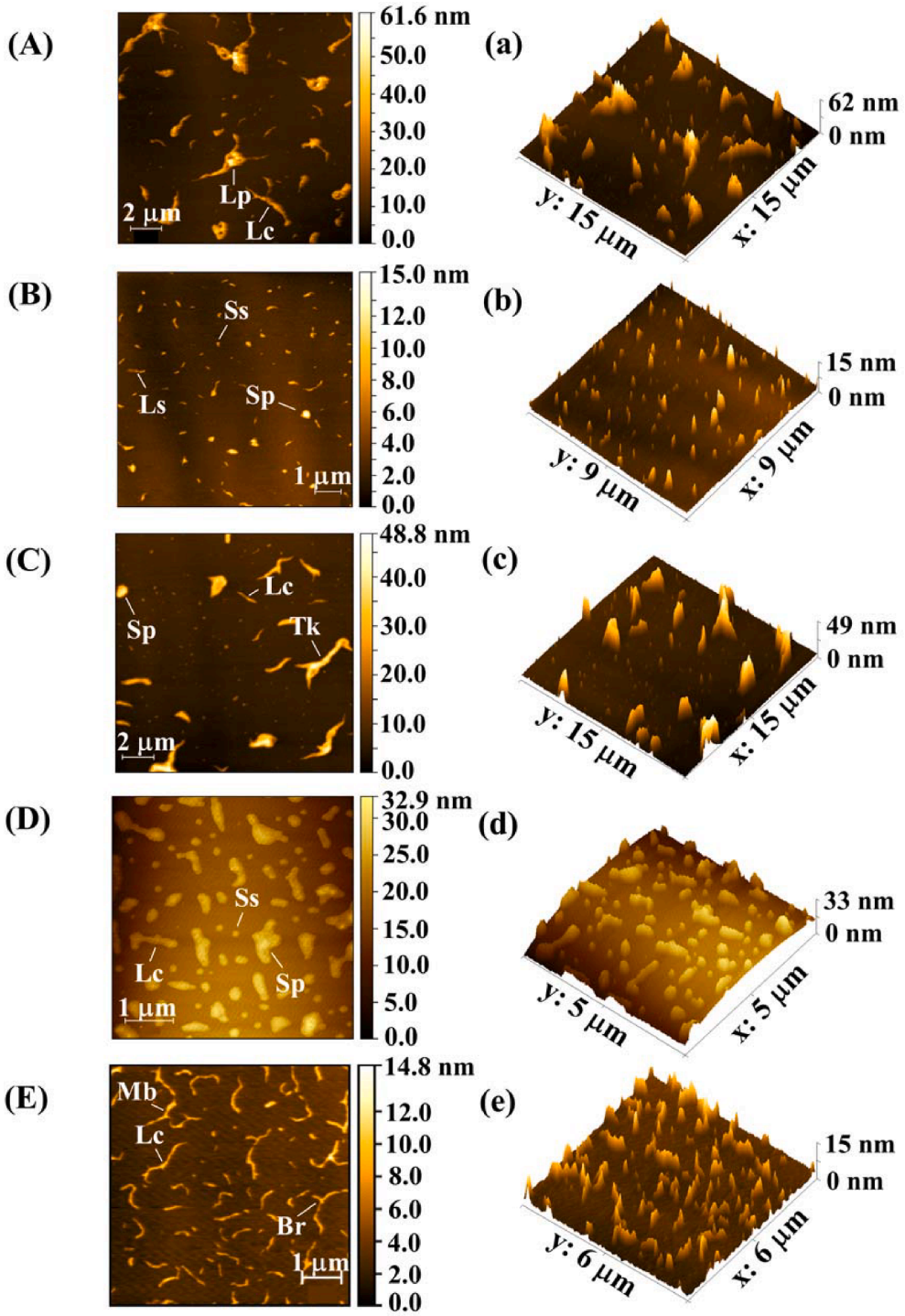

Fig. 4. AFM 2D (first column) and 3D images (second column) of sodium carbonate soluble pectin (SSP) for strawberries after different sanitising treatment. (A and a) Untreatment at day 0; (B and b) DW group at day 7; (C and c) LA group at day 7; (D and d) AEW group at day 7; (E and e) Combined treatment group at day 7. DW: deionised water; LA: levulinic acid; AEW: acidic electrolysed water; Combination: AEW + LA. Note: Lc, long chain; Lp, large polymer; Sp, small polymer; Ss, short straight chain; Ls, loose structure; Tk, thick chain; Br, branched chain; Mb, multiple branched chain.

throughout storage occurred in our study as expected, which was more obvious in samples treated by AEW alone or combined with LA at the later stage of storage (Fig. 2F). Previous studies demonstrated that under reactive oxygen species (ROS) stimulation, the synthesis of ascorbic acid might be activated in fruits, as it could act as an antioxidant to battle with oxidative stress (Hassenberg, Geyer, Ammon, & Herppich, 2011). This might be a reason explaining why AAC in AEW-treated samples could maintain at a higher level during early stage of storage, considering the residual effect of AEW might induce ROS formation in strawberries. However, as the storage time increased, the protective role of ascorbic acid on oxidation process could also lead to its loss, considering its consumption rate in response to the oxidative stress might exceed its synthesis rate, making lower AAC in samples under AEW-included treatments reasonable.

3.4.3. Organic acid content

The changes of strawberries' organic acid content during storage were investigated by using HPLC with malic and citric acid content being mainly focused on, considering these two were identified as the most abundant organic acids in strawberry as reported before (Gündüz and Özdemir, 2014). According to the content shown in Fig. 2G and H, malic acid was the major organic acid in our strawberry samples, different from some previous papers demonstrating that citric acid was dominant, which might be attributed to different cultivars and growth

conditions.

Changes of both organic acids during 7-day cool storage were statistically significant for all treated samples. In general, the level of malic acid in DW- and AEW-treated strawberries remained relatively stable during first 5 days of storage, whereas that in the samples after treatments containing LA were observed with an obvious decrease (Fig. 2G). However, all samples showed an increase in malic acid content on day 7, with no significantly intergroup differences. As for the citric acid content, a downward trajectory was observed in strawberries from all treatment groups over 7 days, with considerable decrease in sanitised samples on day 3 and day 5 particularly compared to that in control samples (Fig. 2H). Previous studies found that tricarboxylic acid (TCA) cycle in strawberry can be affected under various environmental stresses, which might be one reason for decreased level of malic and citric acid shown in current study throughout storage, as these two are mainly produced through TCA cycle and the formation of these TCA intermediates might be very susceptible to LA stimulation (Akhatou, González-Domínguez, & Fernández-Recamales, 2016). Moreover, besides TCA cycle, phosphoenolpyruvate carboxylation reaction can also produce malic acid, which might be upregulated as an adapted strategy to environmental stress, making the increased level of malic acid reasonable at the last stage of shelf-life (Zelle et al., 2008).

3.5. Pectin analysis

As cell wall disassembling and composition changes are highly associated with fruit softening and quality deterioration, the qualitative and quantitative changes of pectins in strawberries were evaluated further in the following sections, to better understand the changes in fruit texture properties after treatments with LA and AEW.

3.5.1. Pectin content analysis

The contents of three kinds of pectin in strawberries on the first and last day of storage are shown in Fig. 3. WSP content did not change significantly in DW and LA + AEW treated samples during storage, whereas it increased a little from 41.6 to 54.5 mg/100 g FW in LA-treated samples and decreased from 118.4 to 99.7 mg/100 g FW in AEW-treated ones. For CSP, its level only decreased slightly (from 107.2 to 83.0 mg/100 g FW) in LA + AEW treated strawberries throughout storage, while that in other three groups remained relatively stable. Distinct changes of SSP content were observed in all treated samples, with an over two-fold increase in control, LA, and LA + AEW group and a nearly five-fold increase in AEW treatment group after 7 days of storage. Overall, treatment with AEW resulted in a significantly higher content of WSP, CSP and SSP than treatment with other methods in our strawberry samples.

The transformations from water-insoluble pectins (e.g., CSP, SSP) to WSP have been regarded as an important cause of fruit softening, as the formers comprise of ionic and covalent ester bonds while the latter is loosely bound in fruit cell walls through non-covalent and non-ionic bonds (Zhu, Huang, Wu, Chen, & He, 2017). In our study, the constant CSP and increased SSP content observed in both single LA and AEW treated strawberries suggested that the degradation of ester bonds might be inhibited over storage period, which might be related to the suppressed pectinolytic enzyme activities under stresses. Although the strawberries in control and other treatment group exhibited similar pectin level at each sampling day, which might be one of the reasons for their similar firmness shown in Section 3.3.1, it did not mean that their pectin structure remained the same. Therefore, the nanostructures of SSP in initial strawberries (without any treatments) on day 0 and the treated strawberries on day 7 were evaluated further by AFM in the following part.

3.5.2. Nanostructure of SSP

Besides the modification of pectin content, the alteration of pectin nanostructures also contributes to the pectin degradation. Considering

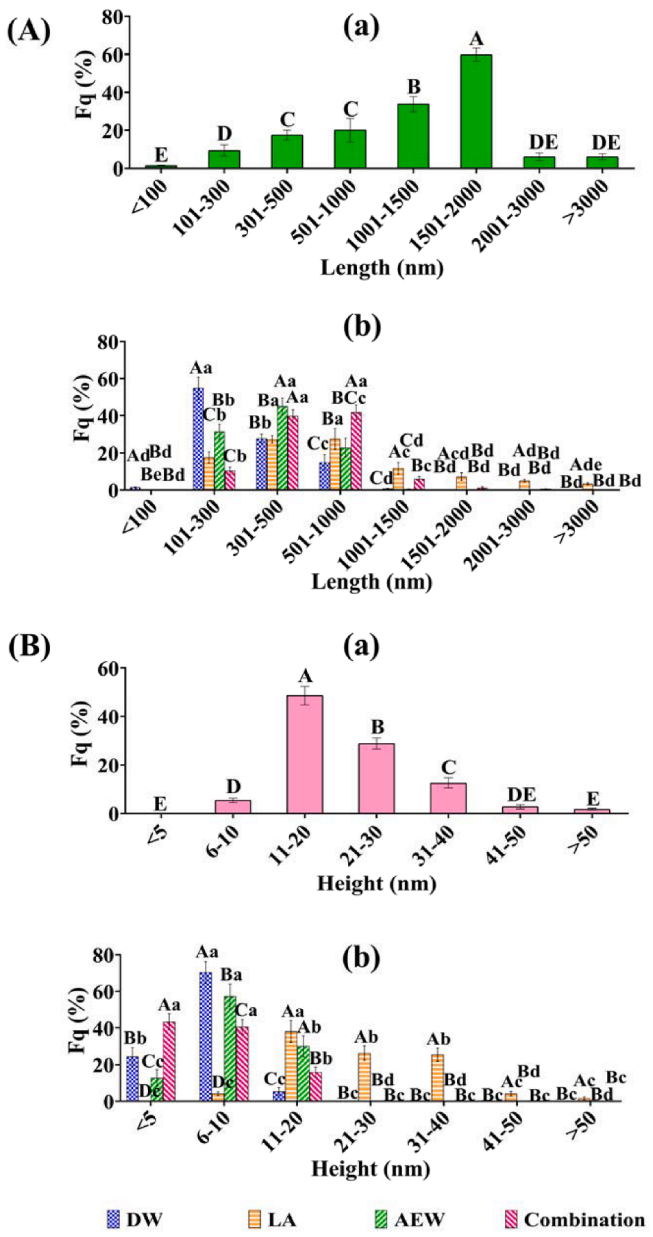

Fig. 5. Quantitative analysis of sodium carbonate soluble pectin (SSP) chains of strawberries. (A) SSP length distribution; (B) SSP height distribution; (a) untreatment group at day 0; (b) each treatment group at day 7. Data are presented as mean values ± standard deviation. For untreatment group at day 0, mean values within same ranges of length (height) with different capital letters are significantly. For each treatment group at day 7, mean values within same ranges of length (height) under different treatments without sharing same capital letters are significantly different; mean values under same treatments in different ranges of length (height) without sharing same lowercase letters are significantly different ($P < 0.05$). DW: deionised water; LA: levulinic acid; AEW: acidic electrolysed water; Combination: AEW + LA; Fq (%): frequency of length (height) value in a particular range.

SSP was reported to be highly correlated with firmness in various produces, such as cheery, honeydew melon, broccoli, and mung bean sprouts (Chen et al., 2018; Liu et al., 2017), the nanoscale morphology of SSP in strawberry under each sanitising treatment was investigated in our study.

AFM images (Fig. 4) from two and three dimensions clearly show the structural changes of SSP during storage. In the untreated samples before storage, long chains (Lc) and large polymer (Lp) structures were observed, indicating heterogeneous and interconnected SSPs in fresh

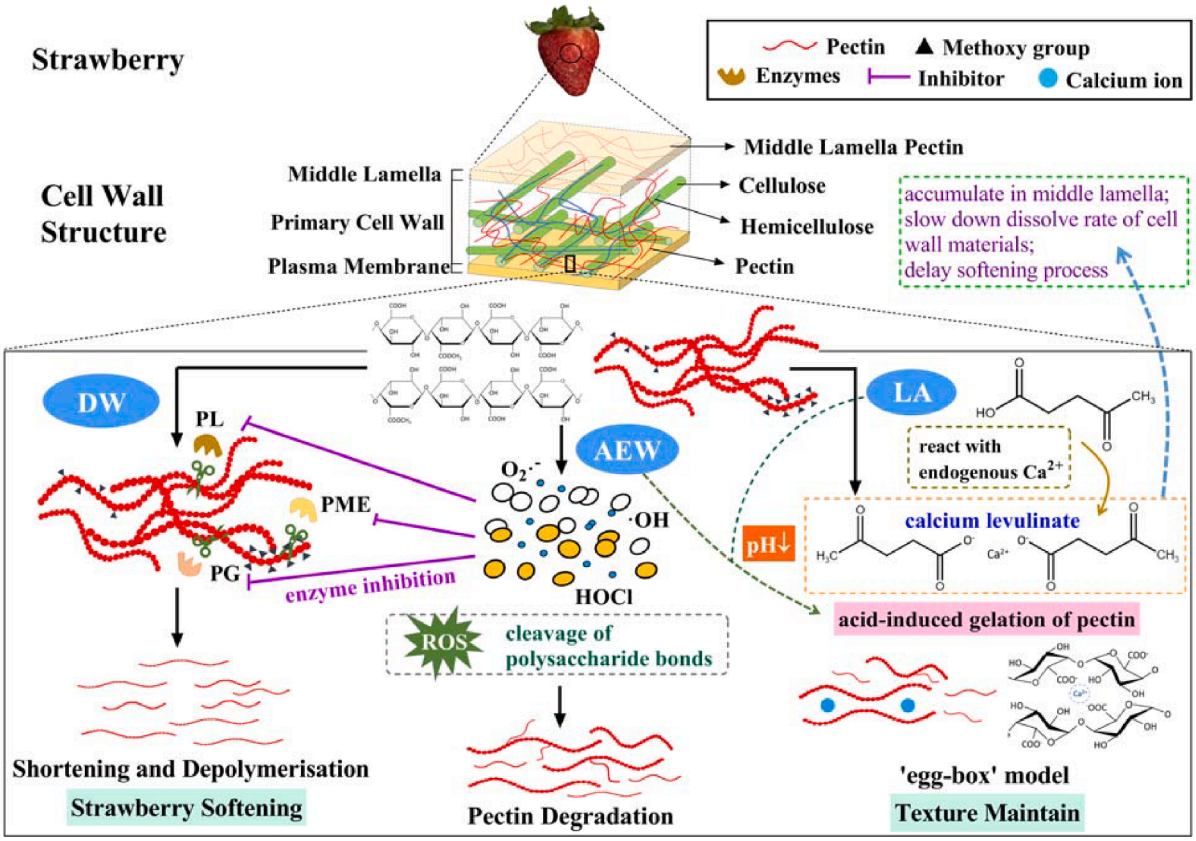

Fig. 6. The schematic illustration of strawberry pectin nanostructural changes after each treatment. DW: Deionised water; AEW: acidic electrolysed water; LA: Levulinic acid; PL: pectate lyase; PG: polygalacturonase; PME: pectin methylesterase; ROS: reactive oxygen species.

strawberries and good maintenance of fruit firmness (Fig. 4Aa). After 7 days of storage, the aggregates were degraded to small polymers (Sp), short straight chains (Ss) and loose structures (Ls) in control group, which were randomly distributed without crosslinking with each other, indicating on-going process of pectin deterioration (Fig. 4Bb). Similar pectin degradation was shown in AEW group, but some long chains and linear single fractions still existed (Fig. 4Dd). On the other hand, treatment containing LA did not accelerate the degradation of SSP in strawberries throughout storage, conversely, thick, long, and branched chains were still noticed in Fig. 4Cc and Ee, indicating good preservation effect of LA on SSP structure in terms of branching and interlocking.

To better understand the modification of SSP chains under each treatment, the length and height values of a bulk of SSP chains at the beginning and last period of storage were measured, and their frequencies in respectively different range were summarised. As shown in Fig. 5Aa, the lengths of SSP in untreated strawberries were mostly within the range of 1501–2000 nm (60%), followed by 1001–1500 nm (33%) and 501–1000 nm (20%). At day 7 (Fig. 5Ab), a remarkable decrease in SSP length was observed in all treatment groups but in different extent, with 55% (DW), 55% (LA), 45% (AEW) and 82% (Combination) of SSP chains within the range of 101–300, 301–1000, 301–500, and 301–1000 nm, respectively. For chain heights (Fig. 5B), most of the untreated strawberry SSP had the values between 11 and 20 nm (49%), followed by 21–30 nm (29%) and 31–40 nm (13%). The height values could be used to determine whether the two chains were overlapped or one chain was a branch of another chain, as the latter exhibited height difference<1.5 times (Chen et al., 2018). After 7 days of storage, a reduction of SSP height was observed in all treated samples as well, with the highest frequency fallen to 6–10 nm in DW and AEW treated strawberries and even to<5 nm in the LA + AEW treated samples, indicating massive decrease in pectin backbone chains (thick chains). However, LA alone did not change the SSP height in strawberries significantly during storage, maintaining most SSP chains at 11–20 nm height as those in untreated group on day 0.

3.5.3. *Hypothetical mechanisms of LA and AEW on pectin nanostructure*

Based on the above observations, a schematic illustration of the variations in strawberry's pectin structure under LA and AEW treatments is shown in Fig. 6. As the most structurally complex cell wall polysaccharides, pectins play important roles in maintaining fruit's firmness and shape. The pectin metabolic enzymes, i.e., pectate lyase (PL), pectin methylesterase (PME), and polygalacturonase (PG), have been regarded as active cell wall degrading enzymes during storage. For example, PG activity in blueberries could increase almost twofold throughout 15-day storage and the increase in PME activity was also observed in sweet cherries during 6 days (Chen et al., 2017; Xin, Jin, Chen, Lai, & Yang, 2020). Considering PG can hydrolyse glycosidic bonds in homogalacturonan, PME can remove the methyl groups from esterified pectin, and PL contributes to the eliminative cleavage of pectate (Wang, Yeats, Uluisik, Rose, & Seymour, 2018), the short SSP chains and loose structures noticed in our control group strawberries could be attributed to a series of active enzymatic actions, followed by pectin depolymerisation and fruit softening.

Although AEW could inhibit the activity of a series of cell wall degrading enzymes as mentioned in Section 3.3.1, which might be attributed to reduced ethylene production during storage under AEW residual effect (Chen et al., 2017), the increased generation of ROS in strawberries which was induced by AEW simultaneously might lead to hydrolytic cleavage of glycosidic bonds, followed by pectin degradation (Chen et al., 2018). This might explain why short SSP chains with lower height of AEW-treated strawberry samples were displayed in AFM images. On the other hand, LA might penetrate the fruit skin and react with endogenous Ca^{2+} to form calcium levulinate, which might accumulate in the middle lamella of cell wall throughout cool storage, slowing down

the dissolution rate of the cell wall materials and delaying softening process (Zhang, Rao, & Wang, 2006). Furthermore, under the combined treatment, since both AEW and LA are acidic compounds, the decrease in pH value could mask the carboxyl groups with negative charge that cause the repulsion of adjacent pectin molecules, which could promote the gelation of soluble pectins for firmness enhancement (Shomer, Frenkel, & Polinger, 1991). Alternatively, it is well known that Ca^{2+} could promote the bonding of adjacent pectin polymers to form a three-dimensional network known as "egg-box" model (Ventura, Jammal, & Bianco-Peled, 2013), thus the formation of these pectin joint structures in strawberries during storage might be induced by the changes of pH, maintaining texture by cell-to-cell bonding.

4. Conclusion

AEW (4 mg/L FAC) and LA (2%, v/v) combination displayed additional antimicrobial effects on natural microbiota of postharvest strawberries than each used alone, with more than 1.11, 1.75 and 1.60 log CFU/g reduction for AMC, APC and yeast and moulds, respectively after 7 days of storage. Moreover, the treatment containing LA could effectively prevent *Salmonella* Typhimurium and *E. coli* O157:H7 growth on strawberries throughout storage, reducing the risk of foodborne illness from fresh produce. Meanwhile, this combined sanitising method did not affect the physicochemical qualities of strawberries significantly when enhancing the microbial safety, maintaining most texture and nutrition indices at an acceptable level. According to AFM analysis, the pectin structure and dimension could be well preserved after this combined treatment, delaying pectin degradation through acid-induced bonding and soluble pectin precipitation. The results suggested that the combination of AEW and LA could be served as a promising technique for perishable fruits' postharvest disinfection, especially in organic food industry. Further technologies such as developing a portable sanitising unit containing our electrolysed water and levulinic acid can be applied in household and food industry to control foodborne pathogens on fresh fruits, but the consumer acceptability of this kind of sanitised fruits should be assessed in further.

CRediT authorship contribution statement

Lin Zhao: Conceptualization, Methodology, Investigation, Software, Visualization, Writing – original draft, Writing – review & editing. **Huixin Li:** Investigation, Writing – original draft. **Ke Wang:** Investigation, Writing – original draft. **Xuan Li:** Investigation. **Chenxi Guo:** Investigation. **Hongshun Yang:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.133533.

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