Carbohydrate Polymers 200 (2018) 427–435

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Combined effects of ultrasound and calcium on the chelate-soluble pectin and quality of strawberries during storage

Lifen Zhang[a], Shunan Zhao[a], Shaojuan Lai[a,b], Fusheng Chen[a,**], Hongshun Yang[c,d,*]

[a] College of Food Science and Technology, Henan University of Technology, Zhengzhou, Henan 450001, PR China
[b] Guangzhou Pulu Medical Technology Co., Ltd, Guangzhou, Guangdong 510800, PR China
[c] Food Science and Technology Programme, c/o Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore
[d] National University of Singapore (Suzhou) Research Institute, 377 Lin Quan Street, Suzhou Industrial Park, Suzhou, Jiangsu 215123, PR China

ARTICLE INFO

Keywords:
Ultrasound
Calcium
Pectin
Atomic force microscopy
Strawberry
Nanostructure

ABSTRACT

The combined effects of ultrasound and calcium on the water migration, quality, and chelate-soluble pectin (CSP) properties of strawberries were investigated using nuclear magnetic resonance (NMR), high-performance liquid chromatography (HPLC), and atomic force microscopy (AFM). The relationship among water migration, firmness, and CSP properties was also determined. Treatment with ultrasound and calcium (U + Ca) prevented the decrease in firmness of strawberries during storage (17 days). Measurements of physicochemical parameters (titratable acidity (TA), soluble solid content (SSC), CSP and Ca content) showed that U + Ca treatment maintained better fruit quality. AFM showed a larger percentage of wider and longer CSP molecules in the U + Ca group (width ≥90 nm; length ≥800 nm). These results, together with the HPLC results, confirmed that U + Ca treatment effectively inhibits CSP degradation. This study revealed that the application of ultrasound and calcium could preserve the quality of stored strawberries.

1. Introduction

Strawberries, with excellent flavour, colour, texture, and nutritional value, can be cultivated in almost all regions of the world. However, to maintain fruit quality in storage during delivery and to prolong seasonal deliveries to markets is a challenge because the fruit are very fragile (Severo, Oliveira, Tiecher, Chaves, & Rombaldi, 2015). To minimise the postharvest decay and maintain the safety, many physical and chemical methods, such as thermal treatment, UV-C radiation, electroysed water, coating, and ultrasound, have been used (Gani et al., 2016; Ramos, Miller, Brandão, Teixeira, & Silva, 2013; Reyes-Avalos et al., 2016; Severo et al., 2017; Vicente, Costa, Martínez, Chaves, & Civello, 2005; Zhang & Yang, 2017). As a non-damaging physical treatment, heat treatment, together with chemical fungicides, can overcome individual shortcomings of postharvest commodities (Zhao et al., 2010). Meanwhile, research indicated that the coating effect is limited because it is difficult to diffuse the coating materials into the interior of the fruit, although this can be promoted by physical technology, such as vacuum impregnation, emulsion and ultrasound (Mao et al., 2017; Sow, Tirtawinata, Yang, Shao, & Wang, 2017; Zhi et al., 2017).

Ultrasound, as a physical technology, has been used to preserve perishable foods because it is effective, safe, non-toxic, and environmentally friendly (Fonteles, Leite, Silva, Fernandes, & Rodrigues, 2017; Tchabo et al., 2017; Wu, Zhang, Jia, Kuang, & Yang, 2018; Yu, Engeseth, & Feng, 2016). When applied to strawberries, it inhibits loss of firmness and helps fruit maintain significantly higher soluble solid content (SSC), titratable acidity (TA), and vitamin C. Aday, Temizkan, Büyükcan, and Caner (2013) reported that ultrasound levels between 30 and 60 W could improve quality and extend the shelf life of strawberries. In addition, researchers found that ultrasound combined with ozone and chlorine dioxide improved factors affecting quality in comparison with individual treatments or control fruit (Aday & Caner, 2014). Combined treatment with ultrasound and acidic electrolysed water inactivated microbes responsible for decay (Zhao, Zhang, & Yang, 2017).

In recent years, researchers have found that the combined treatment of ultrasound and calcium was more effective to enhance the tissue Ca^{2+} content and distribution than either treatment alone (Zhi et al., 2017). Ca^{2+} inactivates the enzyme polygalacturonase (PG), which is

* Corresponding author at: Food Science and Technology Programme, c/o Department of Chemistry, National University of Singapore, Singapore 117543, Singapore.
** Corresponding author.
E-mail address: chmynghs@nus.edu.sg (H. Yang).

https://doi.org/10.1016/j.carbpol.2018.08.013
Received 9 June 2018; Received in revised form 1 August 2018; Accepted 4 August 2018
Available online 07 August 2018
0144-8617/

responsible for the breakdown of cell wall materials and component like pectins, thereby playing a critical role in maintaining fruit quality (Chakraborty, Baier, Knorr, & Mishra, 2015). Ca^{2+} also interacts with the demethylesterified pectin backbones to facilitate the formation of a pectin-Ca^{2+} network, thus enhancing cell wall mechanical properties, which is closely related to fruit texture (Chong, Lai, & Yang, 2015; Day, Xu, Øiseth, & Mawson, 2012; Liu, Tan, Yang, & Wang, 2017; Yang, Wu, Ng, & Wang, 2017; Zhang, Chen, Lai, Wang, & Yang, 2018). Ultrasound combined with Ca^{2+} effectively inhibits the generation of water-soluble and chelate-soluble pectin (CSP) components (Zhi et al., 2017). During fruit softening, calcium (1%) delays changes in the physicochemical properties, particularly the depolymerisation of CSP, which are closely related to fruit firmness (Li, Zhang, Chen, Lai, & Yang, 2018; Liu, Tan et al., 2017).

Strawberries deteriorate in storage primarily because of fungal infections after mechanical bruising and softening. Texture changes are mainly caused by the dissolution of the middle lamella fraction of the cell wall. This process involves pectin solubilisation (Posé et al., 2015). There is evidence that calcium and ultrasound, administered individually or together, can maintain fruit quality in storage, specifically by affecting the pectin structure (Li et al., 2017; Pieczywek, Kozioł, Konopacka, Cybulska, & Zdunek, 2017). However, this treatment has not been widely adopted because the effect of ultrasound combined with calcium on pectin properties is not known. Therefore, the present study aimed to determine the specific effects of treating stored strawberries with a combination of ultrasound and calcium to advance the use of this treatment in commercial facilities.

This study examined the effects of a combined treatment of ultrasound and calcium (U + Ca) on the physicochemical properties of strawberries during storage. Water migration within the fruit, structural properties of CSP, and the relationship of these properties to firmness were investigated using nuclear magnetic resonance (NMR), high-performance liquid chromatography (HPLC), and atomic force microscopy (AFM). The research was designed to verify the efficacy of U + Ca treatment in retaining the quality and prolonging the shelf life of strawberries.

2. Materials and methods

2.1. Materials

Strawberries (*Fragaria* × *ananassa* Duch. cv. 'Sijichun') were harvested at the commercially ripe stage in an orchard two hours away from the laboratory. They were selected for their uniform size and colour, and then randomly divided into four groups, which were treated as follows: immersed in distilled water for 20 min (CK); immersed in 2% calcium chloride solution for 20 min (Ca); treated with ultrasound (40 kHz, 240 W, 20 min, 20 °C) in distilled water (U); treated with ultrasound (40 kHz, 240 W, 20 min, 20 °C) with 2% calcium chloride solution (U + Ca). After treatment, all fruit were stored at 4 °C in a plastic packing box with a height of 9 cm. Every 5 days, 20 fruit from each group were randomly selected and analysed. The experiment was terminated when the decay rate of the fruit reached 50%.

2.2. Firmness

Twenty strawberries from each group were assessed using a TA-XT2i texture analyser (Stable Micro Systems Ltd., Godalming, Surrey, UK) fitted with a P35 probe. Samples were compressed twice to a deformation of 25% with 5 s between strokes. The pre-test, test, and post-test speeds were 3, 1, and 5 mm/s, respectively. The trigger force was 10 g. Twenty replicates from each group were measured individually (Xin, Chen, Lai, & Yang, 2017).

2.3. Weight loss

Weight loss (△m) (%) was determined for 30 strawberries using the following formula:

$$\Delta m = \frac{m_0 - m_1}{m_0} * 100 \quad (1)$$

Where m_1 is the current weight of the fruit and m_0 is the original weight.

2.4. Soluble solids content and titratable acidity

The soluble solids content (SSC) content of 20 fruit from each group was measured using a digital refractometer (Atago Co. Ltd, Tokyo, Japan). The result was expressed as a percentage (%). Titratable acidity (TA) was determined with 0.1 M NaOH using 50 mL diluted juice (50 mL of pressed strawberry juice was diluted to 250 mL with distilled water), and was terminated when the solution colour changed to pink without fading in 30 s. The results were expressed as g of citric acid equivalent per g of fresh weight (FW) (Chen, Tan et al., 2018; Chen, Zhou et al., 2018; Xin et al., 2017).

2.5. Water migration

The water state of the strawberries was measured using VTMR3-010V-T low field nuclear magnetic resonance (NMR) (NiuMag Co. Ltd, Shanghai, China), according to a previous report (Otero & Préstamo, 2009). The operating frequency was 22.6 MHz at 32 °C. To produce samples that would fit into the NMR tube (diameter 10 mm), each strawberry was halved vertically and horizontally into quarters. One such quarter was then longitudinally cut into three anatomical parts designated as: fruit peel, fruit pulp, and fruit core. Each was then put into the core of the radiofrequency (RF) coil and scanned with the multi-pulse echo sequence (Carr-Purcell-Meiboom-Gill (CPMG)). The parameters of the CPMG sequence were as follows: NS = 4, SW = 100 kHz, TD = 55000, NECH = 12,000.

2.6. Calcium content

The calcium content was determined according to our previous report with slight modifications (Mao et al., 2017). Calcium was quantified using an AAS-3000 atomic absorption spectrophotometer (DAFURY Co. Ltd, Beijing, China). Fruit flesh (2.0–4.0 g) was combined with 10 mL of digestion solution (nitric acid:perchloric acid = 4:1) and left at 25 °C to react overnight. The digestion mixture was then made up to a total volume of 25 mL with 20 g/L lanthanum oxide solution.

The Ca content was calculated according to the following formula:

$$X = \frac{(C_1 - C_0) \times V \times f \times 100}{m \times 1000} \quad (2)$$

Where X is the calcium content (mg/100 g fresh fruit), C_1 is the calcium concentration in the sample solution (μg/mL), C_0 is the calcium concentration in the reagent blank (μg/mL), V is the specified volume of the sample (mL), f is the dilution factor, and m is the sample mass (g).

2.7. Chelate-soluble pectin

CSP was extracted according to a previous report with slight modifications (Zhang et al., 2018). Fruit flesh (15 g) was boiled with 200 mL ethanol (80%, v/v) for 20 min, filtered, and cooled to room temperature. The process was repeated twice. The solid residue was then transferred to 50 mL dimethyl sulphoxide (DMSO)/H_2O (9:1 v/v) at 4 °C for 12 h. After filtration, the residue was immersed in 200 mL of chloroform/ethanol solution (2:1 v/v) for 10 min and subsequently washed with 200 mL of acetone until the colour was white. The residue was collected, and designated as cell wall material (CWM). The CWM

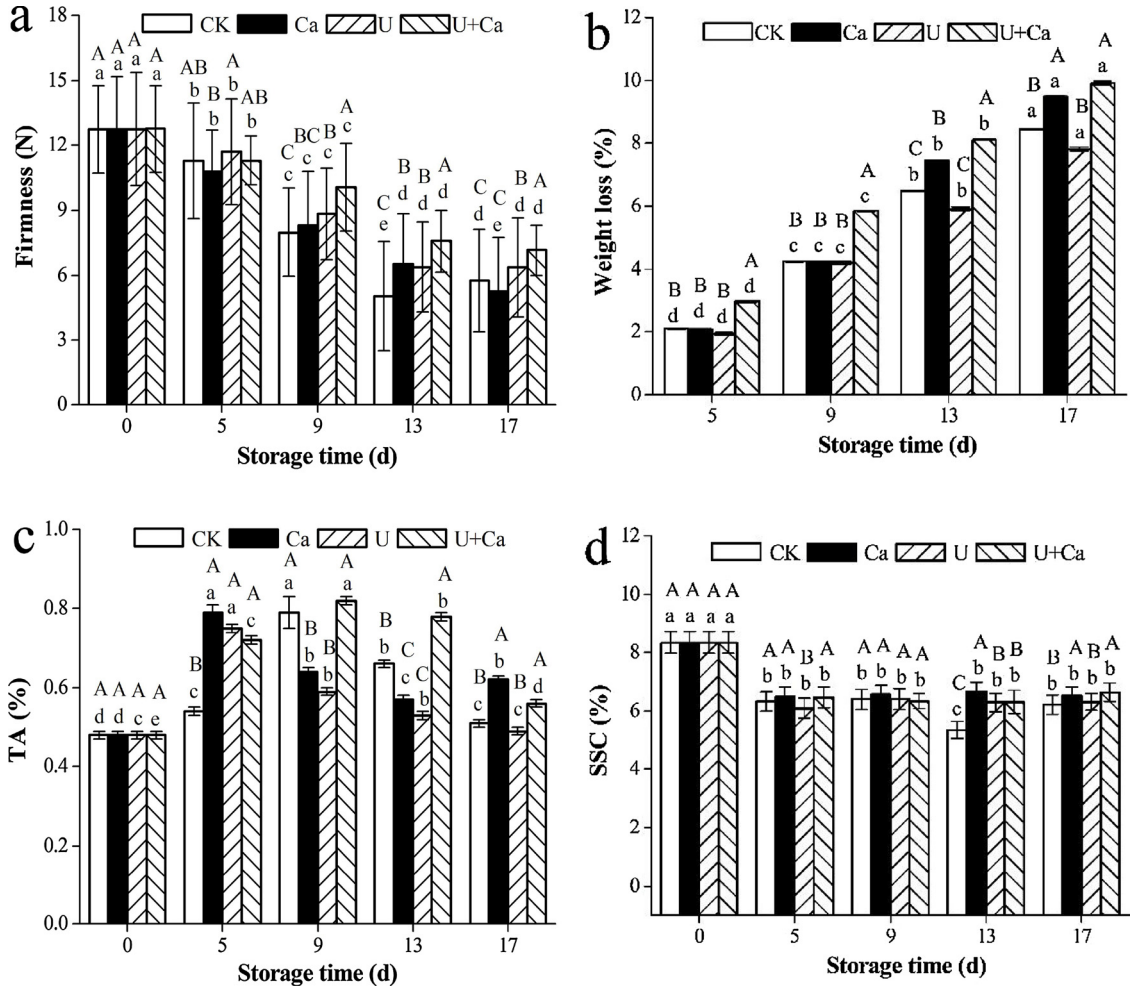

Fig. 1. Combined effects of ultrasound and calcium on the physicochemical properties of strawberries during storage: (a) firmness; (b) weight loss; (c) titratable acidity (TA); (d) soluble solid content (SSC).

Note: CK, control group; Ca, calcium chloride treated group; U, ultrasound treated group; and U + Ca, ultrasound combined with calcium chloride treated group; uppercase superscripts represent significant differences between groups; lowercase superscripts represent significant differences within a group ($P < 0.05$).

was mixed with 10 mL of ultra-purified water and shaken at 25 °C for 4 h, followed by centrifugation at 10,000 × *g*, 4 °C for 10 min (Shanghai Anting Scientific Instrument Factory, Shanghai, China). The above procedure was repeated twice before the residue was resuspended in 10 mL of 50 mM cyclohexane-trans-1,2-diamine tetra-acetate (CDTA) for CSP extraction. After centrifugation (10,000 × *g*, 4 °C for 10 min), the supernatant was collected. The precipitates were subject to repeat extraction twice more, and all supernatants were labelled as CSP. The CSP fractions were determined by carbazole colourimetry with galacturonic acid as the standard.

2.8. Structural analysis of CSP

2.8.1. Monosaccharide constituents

CSP (2 mg) mixed with 2 mL 2 M trifluoroacetic acid (TFA) was hydrolysed at 110 °C for 8 h. A stream of N_2 was used to dry the mixture. The dried sample was then dissolved in 450 μL NaOH (0.3 M) and 450 μL 1-phenyl-3-5-pyrazolone (PMP), and reacted for 30 min at 70 °C in a thermostat-controlled water bath (DC-1006; Ningbo Xinzi Biotechnology Co. Ltd, Ningbo, Zhejiang, China). The solution was then cooled to room temperature and neutralized with 450 μL of HCl (0.3 M). Then chloroform (1.0 mL) was added for extraction. This process was repeated twice. Finally, the solution was filtered through a 0.45 μm membrane and analysed using HPLC (Li et al., 2018).

Mobile phase: Phase A: 15% (v/v) acetonitrile + 0.05 mol/L phosphate buffer solution (sodium dihydrogen phosphate + sodium hydrogen phosphate, pH 6.9); Phase B: 40% (v/v) acetonitrile + 0.05 mol/L phosphate buffer solution (sodium dihydrogen phosphate + sodium hydrogen phosphate, pH 6.9).

Chromatographic conditions: Column: ZORBAX Eclipse XDB-C18 Separation column (4.6 × 250 mm, 5 μm; Agilent Technologies, Inc., Richardson, TX, USA); Detector: PDA 2996 detector (Waters, Milford, MA, USA), detection wavelength: 250 nm, flow rate: l.0 mL/min. Column temperature: 25 °C; time gradient: 0 → 25 → 40 min; concentration gradient: 0 → 15% → 25% phase B; injection volume: 10 μL.

2.8.2. AFM imaging

The nanostructure of CSP was analysed in tapping mode by a NanoScope IIIa AFM (Spm Co. Ltd, Shanghai, China). Pectin solution (5 μL) at a suitable concentration (5–20 μg/mL) was pipetted onto a freshly cleaved mica surface, which was air-dried in a dust-free enclosure before usage. AFM was operated with tapping mode at a scan speed of 2 Hz. The cantilever used was an Si_3N_4 scanner, and the scan rate was 0.5–2 Hz. At least 30 scanned images were captured for each sample.

The width (W) and length (L) of a single strand were calculated using the horizontal distances with offline software (Section Analysis), and the vertical distances were recorded as the height (V). The percentage of pectin chains of particular widths and lengths among all the chains observed was recorded as the frequency (F_q) (Zhang et al.,

2018). At least 90 chains were analysed for each sample to obtain reliable results.

2.9. Statistical analysis

All experiments were performed independently in triplicate. The results were analysed using one-way analysis of variance (ANOVA) using SPSS 20.0 software (IBM, Chicago, IL, USA) and Origin 8.5 (Origin Lab, Hampton, NH, USA). Differences between means were compared using Duncan's test at $P < 0.05$. Thirty parallel imaging tests for pectin were examined by AFM to obtain reliable results.

3. Results and discussion

3.1. Physicochemical properties of strawberries

Texture is a critical characteristic of strawberries related to their quality. Fruit firmness was well retained in the U + Ca and U groups compared with that in CK group between 9–17 days of storage (Fig. 1a). Similar results were reported by Gani et al. (2016). However, there was no obvious difference between the Ca (5.33 N) and CK (5.72 N) groups ($P > 0.05$) at the end of storage (17 days). Compared with that in the other groups, the U + Ca treated fruit had the highest firmness (7.15 N) at the 17th day ($P < 0.05$). Saba reported that Ca^{2+} could maintain and improve the integrity and mechanical properties of the cell wall, inhibiting the softening of fruits (Saba & Sogvar, 2016). In addition, fruit firmness decreased markedly during storage in all groups. Previous studies indicated that this decrease is caused by the degradation of cell walls (Nogata, Ohta, & Voragen, 1993; Vicente et al., 2005; Liu, Chen et al., 2017).

Weight loss in the CK, Ca, U, and U + Ca treated strawberries is shown in Fig. 1b. Significant weight loss in fruit of all treatments was observed during storage (17 days). Samples that had not been treated with calcium lost less weight than those treated with calcium. For example, the weight loss of U + Ca (9.92%) and Ca (9.51%) groups was higher than that of U (7.87%) and CK (8.45%)) groups at the end of storage ($P < 0.05$). The loss of weight in fresh fruit and vegetables is mainly caused by the loss of water (Zhu, Wang, Cao, & Jiang, 2007). Carcel et al. reported that water loss from apple increased by ultrasound treatment (Cárcel, Benedito, Rosselló, & Mulet, 2007). Thus the water loss of strawberries should be further studied.

The variations in TA for the control and treated strawberries during storage are shown in Fig. 1c. All treatments had similar effects on the TA over the whole storage time. TA for all groups slightly increased with storage time. However, a decrease was seen in the control as well as U and U + Ca treated samples over 13 days. This decrease might be caused respiration consumption of organic acids (Tadesse & Abtew, 2016). At the end of storage, the TAs of the Ca and U + Ca groups were significantly higher than those of the CK and U groups ($P < 0.05$). U + Ca treatment inhibited the respiration of fruit.

The SSC of strawberries with different treatments is presented in Fig. 1d. The SSC of all groups decreased at a storage period of 5 days. The SSC remained stable in treated groups on the 13th day of storage. The SSC of the U + Ca group was 6.64%, which was slightly higher than that of the CK group (6.11%) at the end of storage. Sobral et al. reported that higher total soluble solids (TSS) inhibits the decrease of ascorbic acid, and has a positive effect on protection of fruit against oxidation, thus extending the shelf life of fruit (Sobral, Nunes, Maia, Ferreira, & Coimbra, 2017).

3.2. Ca and CSP content

During ultrasound treatment, an increase in the calcium content of the fruit was observed (Fig. 2a). This result was similar to that reported by Mao et al. (2017). Researchers also reported that ultrasound could increase the mass transfer rate between the cell and its extracellular part (Chen, Guo, & Wu, 2016). As shown in Fig. 2a, the Ca content of fresh fruit was 33.74 mg/100 g fresh fruit, while it was 31.93, 41.43, 33.17, and 51.91 mg/100 g fresh fruit for CK, Ca, U, and U + Ca groups at the begin of storage, respectively. The Ca content of the U + Ca group (52.44 mg/100 g fresh fruit) was the highest at 17 days compared with that in the other groups ($P < 0.05$), while no significant difference was found between CK (33.72 mg/100 g fresh fruit) and U group (32.17 mg/100 g fresh fruit) ($P > 0.05$).

As shown in Fig. 2b, the CSP content increased from 93.52 mg/100 g to 238.66, 262.13, 265.41, and 300.82 mg/100 g for the CK, U, Ca, and U + Ca groups, respectively, as measured on day 13 of storage. There is evidence to indicate that the increased CSP is caused by the solubilisation of other pectin fractions rather than increased synthesis of CSP (Zhang, Chen, Zhang, Lai, & Yang, 2017). The CSP content of the U + Ca group was the highest of all groups at the end of storage (17 days). Researchers have reported that the uptake of exogenous calcium ions by strawberries is related to an increase in the fraction of ionically bound pectin, thus promoting the cell-to-cell adhesion and enhancing cell wall stability (Hernández-Muñoz, Almenar, Valle, Velez, & Gavara, 2008; Lara, García, & Vendrell, 2004). A high Ca content, as found in the U + Ca group, is known to inhibit cell wall decomposition (Hernández-Muñoz et al., 2008).

3.3. Monosaccharide constituents of CSP

The monosaccharide constituents of CSP are shown in Table 1. The monosaccharides of CSP consist of mannose (Man), galacturonic acid (GalUA), glucose (Glc), galactose (Gal), rhamnose (Rha), xylnose (Xyl), and arabinose (Ara). The major sugar in CSP is GalUA, as shown in Table 1. This result indicated that the CSP comprises two regions: smooth and hairy. The content of Rha, Gal, and Ara of CSP in the U + Ca-treated groups was higher than that in other groups. The decrease in Rha, Gal, and Ara content indicates the degradation of pectin and directly influences fruit firmness (Tsuchida et al., 2014). The GalUA content of the U + Ca group was the lowest among all the groups. The high content of Glc of the CSP in fresh fruit might be an artefact of the extraction process of the cell wall material.

For pectin molecules, the molar ratio of Rha to GalUA is a parameter that represents the existence and number of rhamnogalactoside-I (RG-I) segments (Liu, Jiang, Yang, & Yang, 2017; Yapo, 2011). At the end of storage, the ratio of Rha to GalUA was 0.10, 0.11, 0.11, and 0.13 for the CK, Ca, U, and U + Ca groups, respectively. In other words, the U + Ca treated fruit contained the most RG-I segments, higher than the fruit treated with either Ca or U alone. Ara and Gal reveal the existence of arabinan and galactan chains in the RG-I region, while the molar ratio of (Ara + Gal) to Rha expresses the branching degree of the RG-I segments. At the end of storage, the molar ratio of (Ara + Gal) to Rha for the control fruit was 7.53, while the ratios for Ca, U, and U + Ca groups were 7.77, 7.86, and 7.93, respectively, suggesting that branching in the RG-I region of control fruit was lower than that in the treated fruit. There was no obvious difference between the molar ratio of (Ara + Gal) to Rha of the U + Ca group and that of fresh fruit. Researchers have consistently reported a close relationship between RG-I regions and fruit firmness (Li et al., 2018). Our results are consistent with those previous reports. This result also indicated that U + Ca treatment inhibits the degradation of CSP during storage.

3.4. Nanostructure of CSP

The morphology of CSP molecules differed in fresh and stored samples (17 days), as shown in Fig. 3. In fresh fruit, CSP molecules mainly contained long chains (Lc), branched (Br), and polymer (P) structures (Fig. 3a). The presence of these structures in the pectin was consistent with similar observations on pectin from strawberries (Posé, Kirby, Mercado, Morris, & Quesada, 2012). In fruit at the end of storage, the CSP molecules of most of the groups comprised short linear

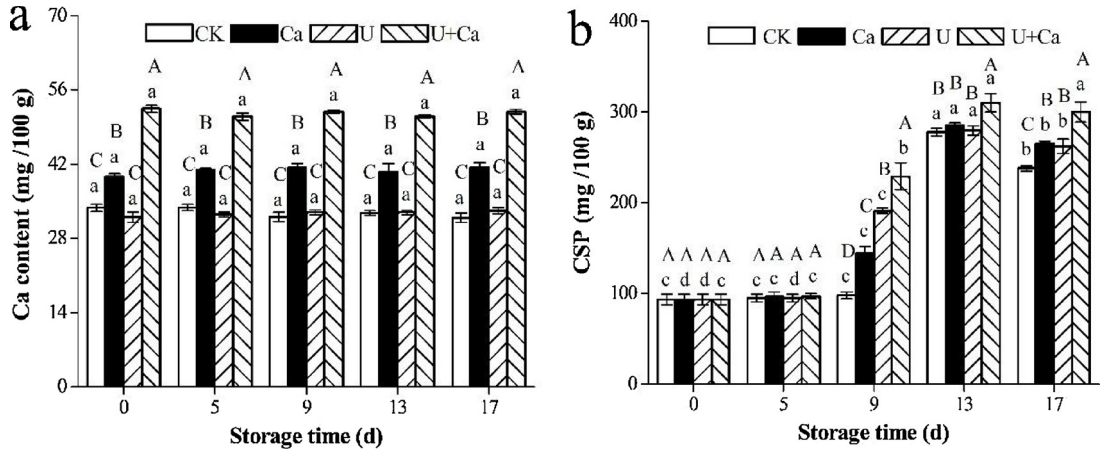

Fig. 2. Combined effects of ultrasound and calcium on the content of calcium (Ca) and chelate-soluble pectin (CSP) in strawberries during storage (a) Ca; (b) CSP. Note: CK, control group; Ca, calcium chloride treated group; U, ultrasound treated group; and U + Ca, ultrasound combined with calcium chloride treated group; uppercase superscripts represent significant differences among groups; lowercase superscripts represent significant differences of different storage time within a group ($P < 0.05$).

chains (Sc) with a small proportion of branches (Fig. 3b–e); some polymers could also be observed (Fig. 3b). Kozioł et al. reported that pectinase degraded CSP molecules and caused the soft texture of fruits (Kozioł, Cybulska, Pieczywek, & Zdunek, 2017). Notably, there were cross-linked structures in the CSP molecules of the Ca and U + Ca treated groups (Fig. 3c and f). Ca^{2+} can interact with pectin *via* non-covalent linkages, and this can inhibit pectin degradation and maintain the firmness of fruit (Kyomugasho, Willemsen, Christiaens, Van Loey, & Hendrickx, 2015; Mao et al., 2017). This result indicated that both Ca and U + Ca treatments effectively inhibited the degradation of CSP molecules at the nano level, based on good cross-linking between calcium and pectin.

The quantitative parameters of CSP molecules were also provided by AFM. Fig. 4a shows the width of CSP molecules of strawberries at the end of storage (17 days). The width of the CSP chains of fresh fruit was mainly in the range of 15–100 nm, with vertical heights around 0.5–3.0 nm. The percentage of CSP of width ≥90 nm in fresh fruit was 70.71%. Degradation of CSP narrowed the molecules during storage according to the increased F_q values of smaller widths for the CK and treated groups. Similar results were observed by Yang et al. (2017). At the end of storage, the F_q value of wider chains (≥90 nm) was 20.62% for the U + Ca group, which was greater than that for the CK (5.73%), U (8.28%), and Ca (10.53%) groups. This result indicated that Ca^{2+} is metabolised during fruit storage. Ca^{2+} cross-linked with demethylesterified pectin decreased the susceptibility of the pectin to depolymerisation, and thus maintained the texture of the fruit (Gwanpua et al., 2017; Kyomugasho et al., 2015).

The length of the CSP chains of fresh fruit was generally in the range of 500–1000 nm (F_q, 70.80%) (Fig. 4b). At the end of storage, the treated groups had a significantly greater frequency of 500–850 nm long CSP chains than did the CK group ($P < 0.05$), and the U + Ca group had the highest F_q (68.57%) in this range. Thus, results for the changes in length and width of CSP chains indicated that U + Ca treatment suppressed CSP molecular degradation.

3.5. Water migration

NMR has the advantage to access molecular information within a food or biological system (Liu, Wu et al., 2017; Liu et al., 2018). The values of NMR measurements for different strawberry tissues are shown in Table 2. The relative signal intensities (I_{21}, I_{22}, and I_{23}) represent bound water, semi-bound water, and free water of the fruit tissues, respectively. At the end of storage (17 days), I_{23} of the peel and pulp of all treated groups decreased compared with that of fresh fruit, while that for the fruit core increased in treated groups, especially the U + Ca group. This indicated that the free water migrates from the pulp to the core of fruit during storage. The U + Ca group had the highest content of free water at their fruit cores, such that these fruits retained a better quality during storage. Researchers have also reported that relaxation times are closely related to fruit weight and sugar content (Geya et al., 2013; Otero & Préstamo, 2009). Fig. 5 shows the T2 relaxation maps, which reflect the morphological features of strawberries, in which brightness corresponds to a high water content. Images of the U + Ca treated strawberries (core) are the brightest, indicating that group

Table 1
Monosaccharide content (mol %) of chelate-soluble pectin (CSP) of fresh strawberries (day 0) and stored samples (day 17).

Monosaccharide (mol %)	Samples				
	Fresh	CK	Ca	U	U + Ca
Man	3.06 ± 0.81^{c}	3.87 ± 0.05^{a}	3.77 ± 0.05^{a}	3.44 ± 0.06^{b}	2.68 ± 0.10^{d}
Rha	3.50 ± 0.21^{d}	4.01 ± 0.11^{b}	4.05 ± 1.11^{b}	3.97 ± 3.17^{c}	4.66 ± 1.18^{a}
GalUA	30.63 ± 0.43^{d}	38.19 ± 0.15^{a}	36.11 ± 2.13^{b}	37.03 ± 2.16^{a}	35.45 ± 3.25^{c}
Glc	27.84 ± 0.22^{a}	16.48 ± 1.37^{b}	15.13 ± 0.19^{d}	15.70 ± 1.12^{c}	12.20 ± 1.21^{e}
Gal	21.69 ± 0.02^{d}	22.77 ± 0.31^{c}	23.78 ± 0.76^{b}	22.49 ± 0.89^{c}	26.72 ± 1.58^{a}
Xyl	7.17 ± 0.18^{d}	7.23 ± 0.19^{d}	9.46 ± 0.15^{a}	8.65 ± 0.05^{b}	8.04 ± 1.31^{c}
Ara	6.11 ± 3.15^{d}	7.45 ± 3.16^{c}	7.70 ± 5.21^{c}	8.72 ± 7.11^{b}	10.25 ± 6.17^{a}
Rha/GalUA	0.11	0.10	0.11	0.11	0.13
(Gal + Ara)/Rha	7.94	7.53	7.77	7.86	7.93

Note: CK, control group; Ca, calcium chloride treated group; U, ultrasound treated group; and U + Ca, ultrasound combined with calcium chloride treated group; Man, mannose; Rha, rhamnose; GalUA, galacturonic acid; Glc, glucose; Gal, galactose; Xyl, Xylose; Ara, arabinose; Different smallcase letters in the same row represent a significant difference at $P < 0.05$.

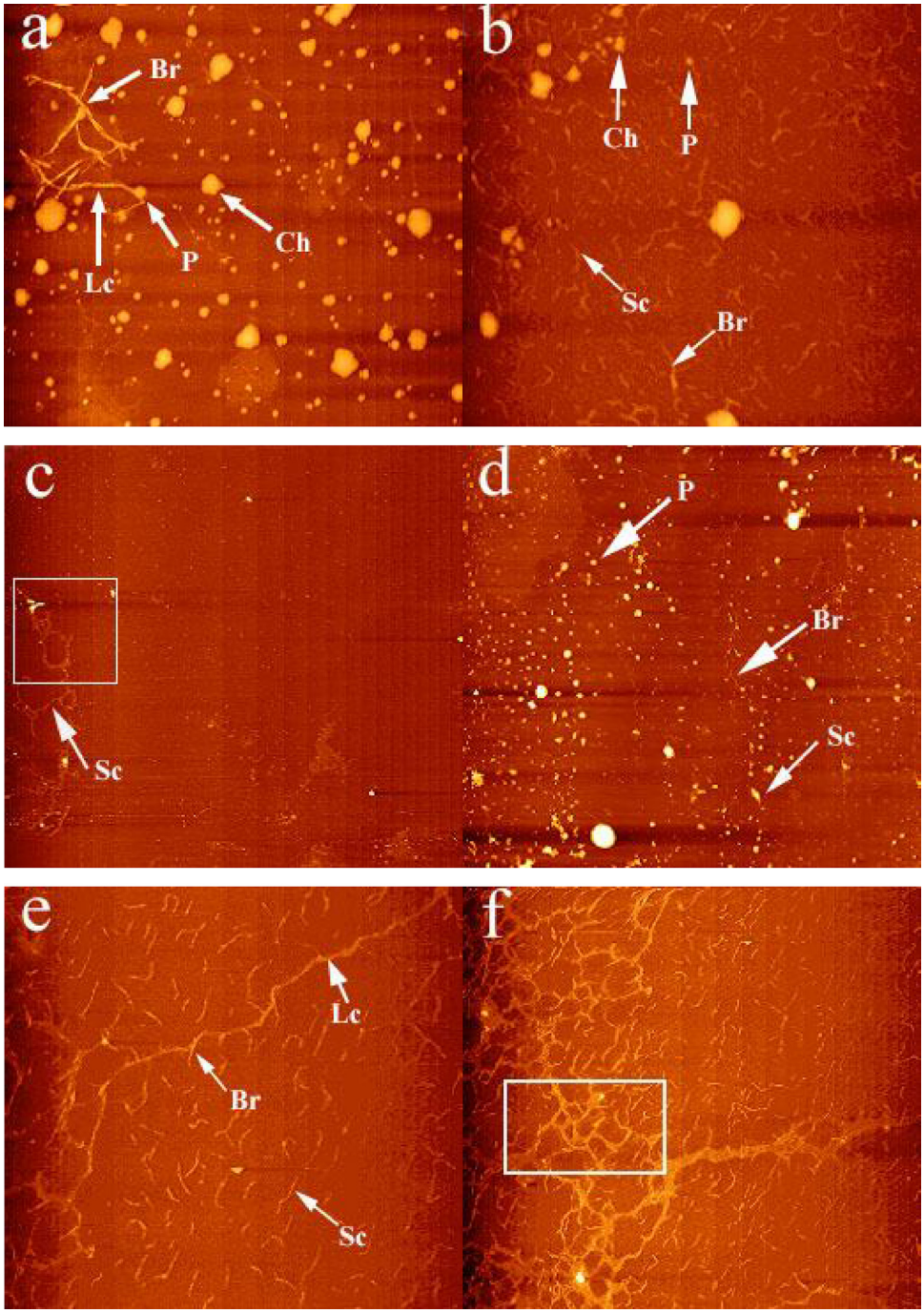

Fig. 3. Atomic force microscopy (AFM) images of chelate-soluble pectin (CSP) molecules of fresh (day 0) and stored strawberries (day 17) (a) fresh fruit; (b) CK; (c) Ca; (d) U; (e,f) U + Ca, scan area: 10.000 × 10.000 μm^2.
Note: Lc, long chain; Br, branch structure; P, polymer; Sc, short chain; Ch, chelating agent; CK, control group; Ca, calcium chloride treated group; U, ultrasound treated group; and U + Ca, ultrasound combined with calcium chloride treated group.

retained the highest amount of water.

3.6. Relationships among water migration, firmness, and CSP

Firmness, as one of the most essential factors affecting fruit quality, is related to the fruit's water content (Fundo et al., 2016). Water influences both the physicochemical and microbiological qualities of food. As shown in Table 2, the free water content in the fruit core increased in the treated groups, especially U + Ca group, compared with that in CK. This result correlates with the firmness results, which showed that treated groups were firmer than the CK group at the end of storage (Fig. 1a). These results suggest a relationship between firmness and water content in the fruit core. Furthermore, the high CSP content also correlated with high firmness and free water content in the U + Ca group. U + Ca treatment effectively maintained strawberries firmness. Previous, similar research in pears reported that changes in hardness relate to changes in water mobility (Fundo et al., 2016).

Fruit softening is one of the main undesirable changes during storage. At the biochemical level, it is caused by cell wall polysaccharide solubilisation and depolymerisation (Billy et al., 2008; Moggia, Graell, Lara, González, & Lobos, 2017). The mechanism by ultrasound combined with calcium chloride inhibits the degradation of CSP is shown in

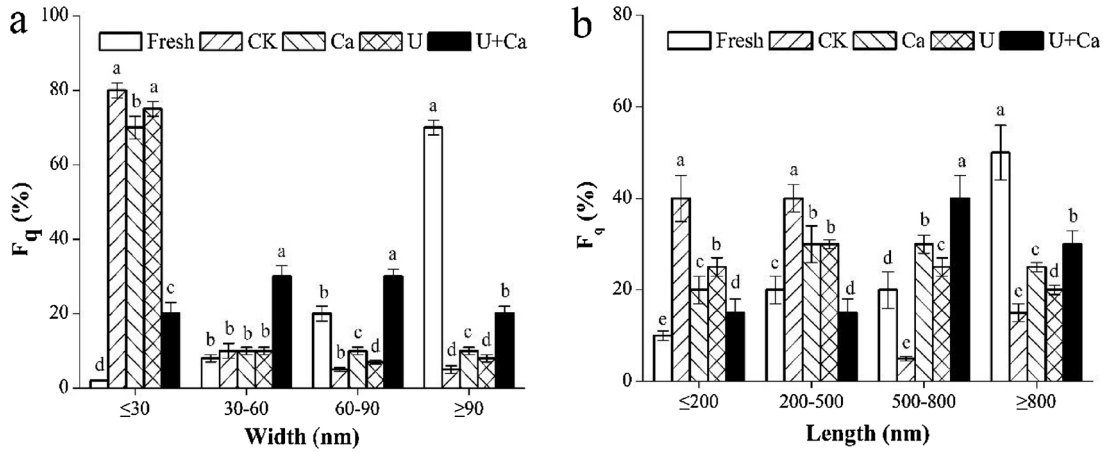

Fig. 4. Quantitative parameters of chelate-soluble pectin (CSP) molecules determined by atomic force microscopy (AFM) (a) Width; (b) Length.
Note: CK, control group; Ca, calcium chloride treated group; U, ultrasound treated group; and U + Ca, ultrasound combined with calcium chloride treated group; 30–60 nm contains 30 but does not contain 60 nm. Different smallcase letters among groups mean significant differences at $P < 0.05$.

Fig. 6. According to Fig. 2, Fig. 6b and d, much more calcium was permeated into strawberry fruit under ultrasound pretreatment. Ca^{2+}, cross-linked with CSP, formed a network structure during storage, and this phenomenon was proven using AFM (Fig. 3c and f). The existence of the Ca^{2+}-CSP network structure inhibited the degradation of CSP. Meanwhile, the quantitative AFM results suggested that the length and width of CSP chains are closely related with strawberry firmness (Figs. 1a and 4 a). Greater values for the width and length of CSP molecules correlated with firmer fruit.

Pectin, one of the major cell wall polysaccharides, plays an essential role in maintaining fruit texture (Zhang et al., 2017). Pectin is a complex heteropolysaccharide, comprising a homogalacturonan region (HG), and type I (RG-I) and type II (RG-II) rhamnogalacturonan regions, among which RG-I is one of the main regions of pectin (Ferreira, Passos, Madureira, Vilanova, & Coimbra, 2015; Yapo, 2011). The side-chain conformation of the RG-I region of pectin also appears to be closely related to the firmness of fruit (Yang, 2014). In the present study, the branching structure of the RG-I region of the CSP in U + Ca group was more extensive; this would contribute to the greater firmness of the fruit in the U + Ca group compared with that of the control fruit during storage (Table 1). Thus, U + Ca treatment can effectively inhibit the softening of strawberries. Previous research also reported that higher resistance to pectinase of CSP correlates with the harder texture and lower susceptibility to postharvest softening of fruit (Kozioł et al., 2017).

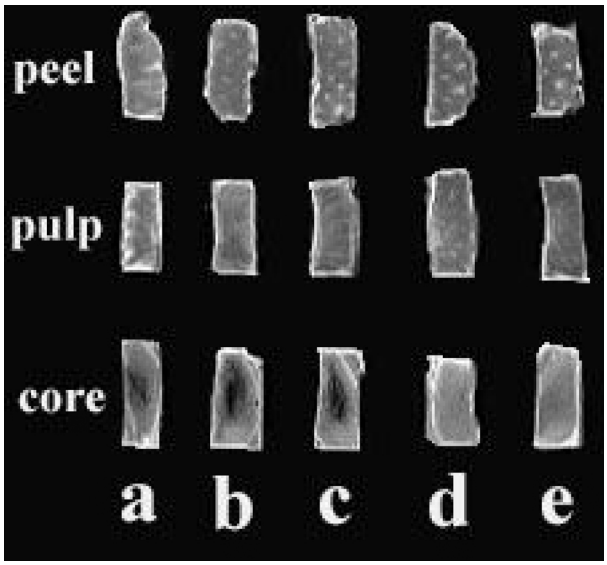

Fig. 5. Nuclear magnetic resonance images of strawberries (a) fresh fruit; (b) CK; (c) Ca; (d) U; (e) U + Ca.
Note: CK, control group; Ca, calcium chloride treated group; U, ultrasound treated group; and U + Ca, ultrasound combined with calcium chloride treated group.

Table 2
Nuclear magnetic resonance (NMR) measurements for different tissues of fresh (day 0) and stored strawberries (day 17).

Fruit tissue	Signal intensity	I (%)				
		Fresh	CK	Ca	U	U + Ca
peel	I_{21}	0.28 ± 0.01^{b}	0.35 ± 0.02^{b}	1.01 ± 0.01^{a}	0.23 ± 0.02^{b}	0.26 ± 0.01^{b}
	I_{22}	0.83 ± 0.01^{b}	1.55 ± 0.01^{a}	1.04 ± 0.02^{a}	0.96 ± 0.01^{b}	1.37 ± 0.01^{a}
	I_{23}	35.13 ± 2.03^{a}	31.72 ± 0.09^{b}	23.51 ± 0.06^{d}	27.26 ± 0.07^{c}	20.68 ± 0.0^{e}
pulp	I_{21}	0.12 ± 0.01^{d}	0.46 ± 0.01^{b}	0.58 ± 0.02^{a}	0.23 ± 0.01^{d}	0.34 ± 0.01^{c}
	I_{22}	0.80 ± 0.01^{b}	0.35 ± 0.01^{d}	0.61 ± 0.01^{c}	0.96 ± 0.03^{a}	1.06 ± 0.01^{a}
	I_{23}	30.86 ± 0.01^{a}	28.78 ± 0.01^{b}	29.94 ± 0.02^{b}	23.06 ± 0.01^{c}	21.84 ± 0.01^{d}
core	I_{21}	0.02 ± 0.01^{d}	0.91 ± 0.02^{a}	0.17 ± 0.01^{c}	0.23 ± 0.02^{b}	0.26 ± 0.01^{b}
	I_{22}	0.70 ± 0.01^{c}	1.62 ± 0.03^{b}	2.25 ± 0.01^{a}	1.88 ± 0.05^{b}	1.63 ± 0.08^{b}
	I_{23}	31.27 ± 1.12^{e}	34.27 ± 1.24^{d}	40.89 ± 2.31^{c}	45.17 ± 2.70^{b}	52.57 ± 3.05^{a}

Note: CK, control group; Ca, calcium chloride treated group; U, ultrasound treated group; and U + Ca, ultrasound combined with calcium chloride treated group; I_{21}, I_{22}, and I_{23} stands for the relative signal intensity of bound water, semi-bound water, and free water, respectively. Different smallcase letters in the same row represent significant difference at $P < 0.05$.

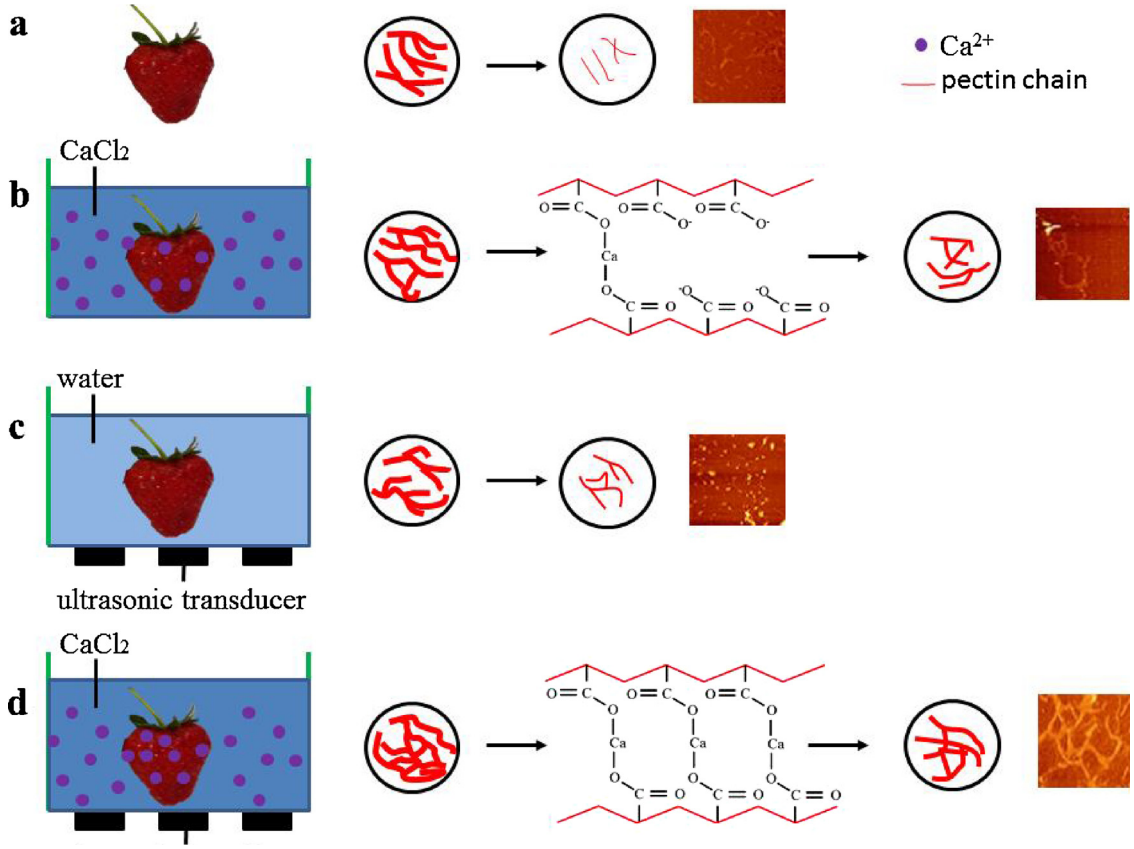

Fig. 6. Mechanism of ultrasound combined with calcium chloride to inhibit the degradation of chelate-soluble pectin (CSP) (a) CK; (b) Ca; (c) U; (d) U + Ca. Note: CK, control group; Ca, calcium chloride treated group; U, ultrasound treated group; and U + Ca, ultrasound combined with calcium chloride treated group.

4. Conclusion

Treating fresh strawberries with a combination of ultrasound and calcium improved their physicochemical properties, internal water migration, and CSP structural properties, during storage. In the U + Ca group, fruit remained firmer, while their SSC, TA, Ca, and CSP contents indicated superior quality. HPLC analysis revealed that there was a larger RG-I region in the CSP molecules of the U + Ca group compared with those in the CK group. Furthermore, the AFM results showed greater width and length of CSP chains in the U + Ca group than in chains of the control group. Therefore, we concluded that U + Ca treatment effectively inhibits the degradation of CSP molecules, which play a dominant role in affecting fruit softening during storage. Future work will explore the application of ultrasound and calcium treatments in preserving other berry fruits during storage.

Acknowledgments

This work was supported by NSFC (31501535 and 31371851), the Singapore Ministry of Education Academic Research Fund Tier 1 (R-143-000-A40-114), Henan Natural Science Foundation of China (162300410046), the Innovation Talent Fund by Henan University of Technology (2014CXRC01) and an industry project from Funlife Food Industry (Chongqing) Co., Ltd (R-143-000-673-597).

References

Aday, M. S., & Caner, C. (2014). Individual and combined effects of ultrasound, ozone and chlorine dioxide on strawberry storage life. *LWT–Food Science and Technology, 57*, 344–351.

Aday, M. S., Temizkan, R., Büyükcan, M. B., & Caner, C. (2013). An innovative technique for extending shelf life of strawberry: Ultrasound. *LWT–Food Science and Technology, 52*, 93–101.

Billy, L., Mehinagic, E., Royer, G., Renard, C. M. G. C., Arvisenet, G., Prost, C., et al. (2008). Relationship between texture and pectin composition of two apple cultivars during storage. *Postharvest Biology and Technology, 47*, 315–324.

Cárcel, J. A., Benedito, J., Rosselló, C., & Mulet, A. (2007). Influence of ultrasound intensity on mass transfer in apple immersed in a sucrose solution. *Journal of Food Engineering, 78*, 472–479.

Chakraborty, S., Baier, D., Knorr, D., & Mishra, H. N. (2015). High pressure inactivation of polygalacturonase, pectinmethylesterase and polyphenoloxidase in strawberry puree mixed with sugar. *Food and Bioproducts Processing, 95*, 281–291.

Chen, Z., Guo, X., & Wu, T. (2016). A novel dehydration technique for carrot slices implementing ultrasound and vacuum drying methods. *Ultrasonics Sonochemistry, 30*, 28–34.

Chen, L., Tan, J. T. G., Pang, X., Yuan, W., Lai, S., & Yang, H. (2018). Energy regulated nutritive and antioxidant properties during the germination and sprouting of broccoli sprouts (*Brassica oleracea var. italica*). *Journal of Agricultural and Food Chemistry, 66*, 6975–6985.

Chen, L., Zhou, Y., He, Z., Liu, Q., Lai, S., & Yang, H. (2018). Effect of exogenous ATP on the postharvest properties and pectin degradation of mung bean sprouts (*Vigna radiata*). *Food Chemistry, 251*, 9–17.

Chong, J. X., Lai, S., & Yang, H. (2015). Chitosan combined with calcium chloride impacts fresh-cut honeydew melon by stabilising nanostructures of sodium-carbonate-soluble pectin. *Food Control, 53*, 195–205.

Day, L., Xu, M., Øiseth, S. K., & Mawson, R. (2012). Improved mechanical properties of retorted carrots by ultrasonic pre-treatments. *Ultrasonics Sonochemistry, 19*, 427–434.

Ferreira, S. S., Passos, C. P., Madureira, P., Vilanova, M., & Coimbra, M. A. (2015). Structure–function relationships of immunostimulatory polysaccharides: A review. *Carbohydrate Polymers, 132*, 378–396.

Fonteles, T. V., Leite, A. K. F., Silva, A. R. A. D., Fernandes, F. A. N., & Rodrigues, S. (2017). Sonication effect on bioactive compounds of cashew apple bagasse. *Food and Bioprocess Technology, 10*, 1854–1864.

Fundo, J. F., Galvis–Sanchez, A., Madureira, A. R., Carvalho, A., Feio, G., Silva, C. L. M., et al. (2016). NMR water transverse relaxation time approach to understand storage stability of fresh–cut 'Rocha' pear. *LWT-Food Science and Technology, 74*, 280–285.

Gani, A., Baba, W. N., Ahmad, M., Shah, U., Khan, A. A., Wani, I. A., et al. (2016). Effect of ultrasound treatment on physico-chemical, nutraceutical and microbial quality of strawberry. *LWT–Food Science and Technology, 66*, 496–502.

Geya, Y., Kimura, T., Fujisaki, H., Terada, Y., Kose, K., Haishi, T., et al. (2013). Longitudinal NMR parameter measurements of Japanese pear fruit during the growing process using a mobile magnetic resonance imaging system. *Journal of Magnetic Resonance, 226*, 45–51.

Gwanpua, S. G., Verlinden, B. E., Hertog, M. L. A. T. M., Nicolai, B. M., Hendrickx, M., &

Geeraerd, A. (2017). A transcriptomics-based kinetic model for enzyme-induced pectin degradation in apple (*Malus* × *domestica*) fruit. *Postharvest Biology and Technology, 130*, 64–74.
Hernández-Muñoz, P., Almenar, E., Valle, V. D., Velez, D., & Gavara, R. (2008). Effect of chitosan coating combined with postharvest calcium treatment on strawberry (*Fragaria* × *ananassa*) quality during refrigerated storage. *Food Chemistry, 110*, 428–435.
Kozioł, A., Cybulska, J., Pieczywek, P. M., & Zdunek, A. (2017). Changes of pectin nanostructure and cell wall stiffness induced in vitro by pectinase. *Carbohydrate Polymers, 161*, 197–207.
Kyomugasho, C., Willemsen, K. L. D. D., Christiaens, S., Van Loey, A. M., & Hendrickx, M. E. (2015). Microscopic evidence for Ca^{2+} mediated pectin-pectin interactions in carrot-based suspensions. *Food Chemistry, 188*, 126–136.
Lara, I., García, P., & Vendrell, M. (2004). Modifications in cell wall composition after cold storage of calcium-treated strawberry (*Fragaria* × *ananassa* Duch.) fruit. *Postharvest Biology and Technology, 34*, 331–339.
Li, N., Chen, F., Cui, F., Sun, W., Zhang, J., Qian, L., et al. (2017). Improved postharvest quality and respiratory activity of straw mushroom (*Volvariella volvacea*) with ultrasound treatment and controlled relative humidity. *Scientia Horticulturase, 225*, 56–64.
Li, Y., Zhang, L., Chen, F., Lai, S., & Yang, H. (2018). Effects of vacuum impregnation with calcium ascorbate and disodium stannous citrate on Chinese red bayberry. *Food and Bioprocess Technology, 11*, 1300–1316.
Liu, Q., Wu, J., Lim, Z. Y., Lai, S., Lee, N., & Yang, H. (2018). Metabolite profiling of *Listeria innocua* for unravelling the inactivation mechanism of electrolysed water by nuclear magnetic resonance spectroscopy. *International Journal of Food Microbiology, 271*, 24–32.
Liu, H., Chen, F., Lai, S., Tao, J., Yang, H., & Jiao, Z. (2017). Effect of calcium treatment and low temperature storage on cell wall polysaccharide nanostructures and quality of postharvest apricot (*Prunus armeniaca*). *Food Chemistry, 225*, 87–97.
Liu, H., Jiang, Y., Yang, H., & Yang, B. (2017). Structure characteristics of an acidic polysaccharide purified from banana (*Musa nana* Lour.) pulp and its enzymatic degradation. *International Journal of Biological Macromolecules, 101*, 299–303.
Liu, Q., Tan, C. S. C., Yang, H., & Wang, S. (2017). Treatment with low–concentration acidic electrolysed water combined with mild heat to sanitise fresh organic broccoli (*Brassica oleracea*). *LWT–Food Science and Technology, 79*, 594–600.
Liu, Q., Wu, J., Lim, Z. Y., Aggarwal, A., Yang, H., & Wang, S. (2017). Evaluation of the metabolic response of *Escherichia coli* to electrolysed water by ^{1}H NMR spectroscopy. *LWT– Food Science and Technology, 79*, 428–436.
Mao, J., Zhang, L., Chen, F., Lai, S., Yang, B., & Yang, H. (2017). Effect of vacuum impregnation combined with calcium lactate on the firmness and polysaccharide morphology of kyoho grapes (*Vitis vinifera* × *V. labrusca*). *Food and Bioprocess Technology, 10*, 609–709.
Moggia, C., Graell, J., Lara, I., González, G., & Lobos, G. A. (2017). Firmness at harvest impacts postharvest fruit softening and internal browning development in mechanically damaged and non-damaged highbush blueberries (*Vaccinium corymbosum* L.). *Frontiers in Plant Science, 8*, 1–11.
Nogata, Y., Ohta, H., & Voragen, A. G. J. (1993). Polygalacturonase in strawberry fruit. *Phytochemistry, 34*, 617–620.
Otero, L., & Préstamo, G. (2009). Effects of pressure processing on strawberry studied by nuclear magnetic resonance. *Innovative Food Science & Emerging Technologies, 10*, 434–440.
Pieczywek, P. M., Kozioł, A., Konopacka, D., Cybulska, J., & Zdunek, A. (2017). Changes in cell wall stiffness and microstructure in ultrasonically treated apple. *Journal of Food Engineering, 197*, 1–8.
Posé, S., Kirby, A. R., Paniagua, C., Waldron, K. W., Morris, V. J., Quesada, M. A., et al. (2015). The nanostructural characterization of strawberry pectins in pectate lyase or polygalacturonase silenced fruits elucidates their role in softening. *Carbohydrate Polymers, 132*, 134–145.
Posé, S., Kirby, A. R., Mercado, J. A., Morris, V. J., & Quesada, M. A. (2012). Structural characterization of cell wall pectin fractions in ripe strawberry fruits using AFM. *Carbohydrate Polymers, 88*, 882–890.
Ramos, B., Miller, F. A., Brandão, T. R. S., Teixeira, P., & Silva, C. L. M. (2013). Fresh fruits and vegetables–An overview on applied methodologies to improve its quality and safety. *Innovative Food Science & Emerging Technologies, 20*, 1–15.
Reyes-Avalos, M. C., Femenia, A., Minjares–Fuentes, R., Contreras–Esquivel, J. C., Aguilar-González, C. N., Esparza-Rivera, J. R., et al. (2016). Improvement of the quality and the shelf Life of figs (*Ficus carica*) using an alginate-chitosan edible film. *Food and Bioprocess Technology, 9*, 1–11.
Saba, M. K., & Sogvar, O. B. (2016). Combination of carboxymethyl cellulose-based coatings with calcium and ascorbic acid impacts in browning and quality of fresh-cut apples. *LWT – Food Science and Technology, 66*, 165–171.
Severo, J., Oliveira, I. R. D., Tiecher, A., Chaves, F. C., & Rombaldi, C. V. (2015). Postharvest UV-C treatment increases bioactive, ester volatile compounds and a putative allergenic protein in strawberry. *LWT–Food Science and Technology, 64*, 685–692.
Severo, J., Oliveira, I. R. D., Bott, R., Bourvellec, C. L., Renard, C. M. G. C., Page, D., et al. (2017). Preharvest UV-C radiation impacts strawberry metabolite content and volatile organic compound production. *LWT–Food Science and Technology, 85*, 390–393.
Sobral, M. M. C., Nunes, C., Maia, A., Ferreira, P., & Coimbra, M. A. (2017). Conditions for producing long shelf life fruit salads processed using mild pasteurization. *LWT–Food Science and Technology, 85*, 316–323.
Sow, L. C., Tirtawinata, F., Yang, H., Shao, Q., & Wang, S. (2017). Carvacrol nanoemulsion combined with acid electrolysed water to inactivate bacteria, yeast *in vitro* and native microflora on shredded cabbages. *Food Control, 76*, 88–95.
Tadesse, T. N., & Abtew, W. G. (2016). Effect of hot water treatment on reduction of chilling injury and keeping quality in tomato (*Solanum lycopersicum* L.) fruits. *Journal of Stored Products and Postharvest Research, 7*, 61–68.
Tchabo, W., Ma, Y., Kwaw, E., Zhang, H., Li, X., & Afoakwah, N. A. (2017). Effects of ultrasound, high pressure, and manosonication processes on phenolic profile and antioxidant properties of a sulfur dioxide-free mulberry (*Morus nigra*) wine. *Food and Bioprocess Technology, 10*, 1210–1223.
Tsuchida, Y., Yakushiji, H., Oe, T., Negoro, K., Gato, N., Kotani, T., et al. (2014). Differences in cell-wall polysaccharide degradation during softening process in two cultivars of Japanese apricot fruits. *The Japanese Society for Horticultural Science, 83*, 81–89.
Vicente, A. R., Costa, M. L., Martínez, G. A., Chaves, A. R., & Civello, P. M. (2005). Effect of heat treatments on cell wall degradation and softening in strawberry fruit. *Postharvest Biology and Technology, 38*, 213–222.
Wu, Q., Zhang, X., Jia, J., Kuang, C., & Yang, H. (2018). Effect of ultrasonic pretreatment on whey protein hydrolysis by alcalase: Thermodynamic parameters, physicochemical properties and bioactivities. *Process Biochemistry, 67*, 46–54.
Xin, Y., Chen, F., Lai, S., & Yang, H. (2017). Influence of chitosan-based coatings on the physicochemical properties and pectin nanostructure of Chinese cherry. *Postharvest Biology and Technology, 133*, 64–71.
Yang, H. (2014). *Atomic force microscopy (AFM): Principles, modes of operation and limitations.* New York: Nova Science Publishers, Incorporated.
Yang, H., Wu, Q., Ng, L. Y., & Wang, S. (2017). Effects of vacuum impregnation with calcium lactate and pectin methylesterase on quality attributes and chelate-soluble pectin morphology of fresh-cut papayas. *Food and Bioprocess Technology, 10*, 901–913.
Yapo, B. M. (2011). Rhamnogalacturonan-I: A structurally puzzling and functionally versatile polysaccharide from plant cell walls and mucilages. *Polymer Reviews, 51*, 391–413.
Yu, J., Engeseth, N. J., & Feng, H. (2016). High intensity ultrasound as an abiotic elicitor–effects on antioxidant capacity and overall quality of romaine lettuce. *Food and Bioprocess Technology, 9*, 262–273.
Zhang, J., & Yang, H. (2017). Effects of potential organic compatible sanitisers on organic and conventional fresh-cut lettuce (*Lactucasativa* Var. *Crispa* L.). *Food Control, 72*, 20–26.
Zhang, L., Chen, F., Lai, S., Wang, H., & Yang, H. (2018). Impact of soybean protein isolate–chitosan edible coating on the softening of apricot fruit during storage. *LWT–Food Science and Technology, 96*, 604–611.
Zhang, L., Chen, F., Zhang, P., Lai, S., & Yang, H. (2017). Influence of rice bran wax coating on the physicochemical properties and pectin nanostructure of cherry tomatoes. *Food and Bioprocess Technology, 10*, 349–357.
Zhao, L., Zhang, Y., & Yang, H. (2017). Efficacy of low concentration neutralised electrolysed water and ultrasound combination for inactivating *Escherichia coli* ATCC 25922, *Pichia pastoris* GS115 and *Aureobasidium pullulans* 2012 on stainless steel coupons. *Food Control, 73*, 889–899.
Zhao, Y., Tu, K., Tu, S., Liu, M., Su, J., & Hou, Y. (2010). A combination of heat treatment and *Pichia guilliermondii* prevents cherry tomato spoilage by fungi. *International Journal of Food Microbiology, 137*, 106–110.
Zhi, H., Liu, Q., Xu, J., Dong, Y., Liu, M., & Zong, W. (2017). Ultrasound enhances calcium absorption of jujube fruit by regulating the cellular calcium distribution and metabolism of cell wall polysaccharides. *Journal of the Science of Food and Agriculture, 97*, 5205–5210.
Zhu, X., Wang, Q., Cao, J., & Jiang, W. B. (2007). Effects of chitosan coating on postharvest quality of mango (*Mangifera indica* L. cv. Tainong) fruits. *Journal of Food Processing and Preservation, 32*, 770–784.