Quality attributes and cell wall properties of strawberries (Fragaria annanassa Duch.) under calcium chloride treatment

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Abstract

Effects of CaCl₂ (0%, 1% and 4%) treatment on quality attributes and cell wall pectins of strawberry fruits stored at 4°C for 15 d were investigated. Strawberry firmness was not significantly affected by CaCl₂ treatment. Compared to the other groups, the 1% CaCl₂ group had better quality attributes, including decay rate, weight loss and soluble solids content. The treatment with 4% CaCl₂ inhibited weight loss but caused phytotoxicity. During storage, the chain widths and lengths of water-soluble pectin (WSP), chelate-soluble pectin (CSP) and sodium carbonate-soluble pectin (SSP) decreased. Strawberry softening seemed to be due to modifications of CSP and SSP, especially the side chains. CaCl₂ treatment significantly slowed the breakdown of CSP and SSP chains by strengthening the ionic crosslinkages among these pectin molecules. These results illustrate the fundamental CaCl₂ effects and will help improve the application of CaCl₂ to postharvest fruits.

1. Introduction

Strawberry (Fragaria annanassa Duch.) is highly appreciated for its excellent organoleptic properties. It is harvested at full maturity and has a short shelf life due to its soft texture and high softening rate. Therefore, it is very necessary to understand the fundamentals of postharvest strawberry softening and find ways to extend its shelf life.

The main factor determining postharvest putridness of fruit is the rate of fruit softening. The mechanisms by which fruits soften during storage remain unclear. Softening is partly due to turgor loss, starch degradation (Ali, Chin, & Lazan, 2004) and chemical modifications in the cell wall (Vicente, Saladié, Rose, & Labavitch, 2007). During cold storage, changes of fruit composition and the structure of cell walls affect fruit softening (Brummell, Cin, Lurie, Crisosto, & Labavitch, 2004; Rosili, Civello, & Martínez, 2004). Fruit softening has a close relationship with the depolymerisation and solubility of flesh cell wall polysaccharides (Brummell et al., 2004). Overall, changes in the cell wall involve an increase in water-soluble pectin and a trend of decrease in chelate-soluble pectin and loss of galactose, arabinose and uronic acid residues (Brummell et al., 2004; Lara, García, & Vendrell, 2004).

Calcium is an element affecting fruit quality and preservation. Cell wall calcium plays a fundamental role in maintaining cell wall stabilization and integrity by interacting with the uronic acid carboxyl functions in pectin polysaccharide chains to create the so-called pectin ‘egg-box’ (Vicente et al., 2007). Calcium was utilised for harvested fruits in maintaining qualities, preventing softening, reducing the rate of roteness and prolonging shelf life (Verdini, Zoggilla, & Rubiolo, 2008). These fruits include apples (Siddiqui & Bangerth, 1993), apricots (Antunes, Correia, Miguel, Martins, & Neves, 2003; Liu et al., 2009; Souty et al., 1995), peaches (Manganaris, Vasilakakis, Diamantidis, & Mignani, 2007) and strawberries (Lara et al., 2004; Rosen & Kader, 1989; Verdini et al., 2008). Calcium treatment also reduces pectin solubility (Lara et al., 2004), strengthens cell walls (Vicente et al., 2007), delays fruit ripening and decreases decay rate (Lara et al., 2004) and maintains firmness (García, Herrera, & Morilla, 1996). However, there are no direct ultrastructural studies indicating the mechanism of calcium effects. The application of calcium treatment in specific fruits only relies on trial and error.

Although there are many reports about the changes of pectin content and chemical composition (Kurz, Carle, & Schieber, 2008; Lara et al., 2004; Missang, Renard, Baron, & Drilleau, 2001), it is believed that physical structure, rather than chemical composition,
the plant cell wall pectin, determines the protective effects of the cell wall materials. In addition, conventional sugar analysis is a known technique providing some information without considering the branch structure of pectin (Round et al., 2001).

Atomic force microscopy (AFM) can characterise the heterogeneity of pectin polysaccharides of fruits. The technique has been used for qualitative description (Round et al., 2001), and quantitative analysis of pectin molecules (Kirby, MacDougall, & Morris, 2008; Liu et al., 2009; Yang, Feng, An, & Li, 2006). Application of AFM in investigating the nonstructural changes of cell wall pectin of strawberry, after CaCl₂ treatment during storage, would help to illustrate the effects of calcium on postharvest fruits.

The objective of this study was to investigate the fundamental of CaCl₂ effects on postharvest strawberry during storage. Qualitative and quantitative changes of three kinds of pectins, and the physicochemical properties of strawberry, were analysed. The results help to illustrate the detailed effects of calcium on the three kinds of pectins and related physicochemical properties of stored strawberry.

2. Materials and methods

2.1. Fruit material

‘Shijixiang’ strawberries, at mature stage, harvested early in the morning in April from an orchard in Zhongmou, Zhengzhou, China, were transported for 40 km to a laboratory in a refrigerated truck 2 h after harvest. The fruits were selected according to their size (medium), ripening stage (firm inside with three-quarters of their colour red) and presence of stalks. About 2000 fruits (about 24 kg) were selected for experiment. Twenty strawberries were washed and used for initial firmness analysis and pectin extraction. The others were randomized and divided into three treatment groups and each group was counted and weighed before the following three treatments: distilled water (CK group), 1% CaCl₂, and 4% CaCl₂. The treatments were performed by placing the fruits in 10 l of CaCl₂ solution or distilled water for 15 min. After allowing the fruits to dry at ambient temperature (25 °C) for 1 h, fruits were stored at 4 °C in a temperature-controlled chamber. 5, 10 and 15 d after harvesting, 20 fruits of each lot were randomly removed from storage and used for analyses, except for decay rate and weight loss. The fruits were allowed to stand at 25 °C for about 3 h before starting the test. Separate lots of fruits were used for determining decay rate and weight loss, respectively. Each treatment was performed in triplicate.

2.2. Decay rate, weight loss, firmness, titratable acidity, soluble solids content, and pH

At each sampling, the number of decayed fruits relative to the initial amount of fruits per each lot (100 fruits) was counted as fruit decay rate. For determining weight loss, at each sampling, lots with fruits (20 per each lot) were taken out from cold storage and placed at room temperature for 3 h. Individual fruit of each lot were weighed and calculated using the equation: Weight loss (%) = \( \frac{(m - m₀)}{m₀} \times 100 \), in which \( m ⟹ m₀ \) mean the individual weight of each fruit at present and originally, respectively.

The firmness of samples was measured using a TA-XT2i texture analyser (Stable Micro Systems Ltd., Godalming, Surrey, UK) according to Colla, Sobral, and Menegalli (2006) with the parameters as: load cell = 25 kg, probe = 100 mm diameter aluminium cylinder, test speed = 1 mm/s, deformation = 75%. Firmness was defined as the maximum penetration force (N) reached during tissue breakage. Strawberries of uniform size, from which the calyces had been removed to obtain even surfaces, were used to determine the break force. Per treatment, 10 fruits were used for each storage time. The firmness of the 10 samples in each treatment was measured individually.

Titratable acidity (TA), soluble solids content (SSC) and pH were assessed with juice obtained from 20 fruits per treatment. TA, expressed as percent citric acid, was measured with well-mixed juice titrated with 0.1 M NaOH, using a titrometer. SSC, expressed as Brix, was determined by a refractometer (WVT-J, Sichuan, China), and pH of the solution was determined by a potentiometer, using 5 ml of fruit juice diluted in 50 ml distilled water (Liu et al., 2009).

2.3. Cell wall polysaccharides extraction and content

Cell wall materials (CWM) were extracted in triplicate with each sampling (10 fruits per extraction) according to the method of Zhou et al. (2000). Fresh peeled flesh (10 g) was ground before being boiled three times in ethanol (80%, v/v) (Tianjin Resent Chemical Co. Ltd., China) for 20 min; then ethanol was decanted by filtration, and the solid residue was transferred to 50 ml of dimethyl sulphoxide (DMSO, Tianjin Resent Chemicals Co. Ltd., China): \( \text{H}_2\text{O} (9:1, v/v) \) for 12 h at 4 °C. After filtration, the residue was dipped for 10 min in a solution of 2:1 chloroform (Suzhou Chemicals Co. Ltd., China)–ethanol (v/v) and subsequently washed in acetone. The materials recovered by filtration were weighed as yield of CWM. Different cell wall polysaccharide fractions were obtained by stepwise extraction with respect to their differences in solubility. The CWM from each sample was isolated with 10 ml of ultra purified water for 4 h at 25 °C, and then centrifuged at 10,000 g at 4 °C for 10 min. The supernatant was collected and the remaining pellet was subject to reiteration extraction twice more. Then the three supernatants were combined as water-soluble pectin (WSP). The residue was then extracted in 10 ml of 50 mM cyclohexane-trans-1,2-diamine tetra-acetate (CDTA) (Tianjin Zinco Fine Chemical Institute, China) three times, to fractionate chelate-soluble pectin (CSP). The residue, after CSP extraction, was further extracted with 10 ml of 50 mM/2 mM Na₂CO₃/CDTA, three times, to get sodium carbonate-soluble pectin (SSP). Total sugar in each of the three pectin fractions was analysed by the carbazole–sulphuric acid assay at 540 nm (Liu et al., 2009).

Pectin solution (2 ml) was blended with 12 ml of sulphuric acid (98% w/w) (Luoyang Chemicals Co. Ltd., China) in a test tube and cooled using tap water; then the mixture was boiled for 10 min and cooled again. Carbazole (Tianjin Kermel Chemical Reagent Co. Ltd., China) ethanol solution (0.5 ml) was added to the solution and the whole was incubated for 30 min at room temperature. The absorbance at 540 nm was measured using a UV-2000 spectrophotometer (Unico Instrument Co. Ltd., Shanghai, China) with galacturonic acid (Sigma–Aldrich Co. Ltd., St. Louis, MO, USA) as standard. The results were presented as galacturonic acid equivalents (GAE), expressed per 100 g fresh mass (FM) (Keutgen & Pawelzik, 2007).

2.4. AFM analysis

The AFM of WSP, CSP and SSP was conducted according to the procedure used previously (Kirby et al., 2008; Yang et al., 2006). About 10 µl of diluted pectin solution of reasonable concentration (about 10 µg/ml) was dropped onto a freshly cleaved mica sheet surface. The mica was dried in air at room temperature. Tapping mode was carried out using a multimode NanoScope IIIa AFM (Vecco Metrology Group, Digital Instruments, CA, USA). The cantilever used was a Si₃N₄ scanner with resolution of 0.1 nm in vertical and 1–2 nm in horizontal positions. The scan rate was about 0.5–2 Hz.
The AFM images were analysed offline with AFM software (Version 5.30r3sr3). The images were sectioned along a line orthogonal to the direction at which the samples were obtained, and the surface profiles of the sections were then plotted. The horizontal and vertical distances of the chains were recorded as width \((W)\) and height \((V)\), respectively. Number of particular chain widths (or range of lengths) determined by AFM was recorded as frequency \((Fq)\). The length \((L)\) of single chain was determined according to horizontal distance. The height of the chains was used to determine whether the chains were branches or lapped over of two single chains. At least 10 images were examined for each sample for obtaining reliable statistical results.

Fig. 1. Effects of CaCl\(_2\) treatment on physiochemical properties and pectin contents of strawberries during storage.
2.5. Statistical analysis

The data were analysed with the statistical programmes SPSS for Windows, 13.0 standard version (SPSS Inc., Chicago, IL, U.S.). Analysis of variance (ANOVA) was performed to determine the effects of storage time and CaCl$_2$ treatment on physicochemical properties, as well as nanostructure of pectins from AFM images. Pearson’s correlation analysis, among the parameters, was performed. Duncan’s test was performed to examine differences among different groups. Comparisons that yielded $P < 0.05$ were considered significant.

3. Results and discussion

3.1. Firmness and physicochemical properties

Fruit firmness is the most important parameter reflecting storage characteristics of fruits. Fig. 1A shows the firmness of strawberries with different CaCl$_2$ treatment and storage time. During storage, firmness of strawberries decreased slowly at first. However, during the 5th to 10th day of storage, reduction of firmness became much faster and, interestingly, then the firmness increased to a level even greater than that at harvest, which was also observed by others (Lara et al., 2004). The phenomenon was induced by the loss of water content in fruit cells, which resulted in an increase of force needed to break up the fruit peel. As shown in Figure 1A, CaCl$_2$ treatment did not affect the fruit firmness significantly, with no significant differences among different treatments. The tips and calyces of strawberries decayed more easily than did the equatorial parts. Therefore, the limiting factor of firmness was from the tips and calyces and firmness was calculated from the tips and calyces of the strawberries (Hernández-Muñoz, Almenar, Valle, Velez, & Gavara, 2008; Pelayo, Ebeler, & Kader, 2003). Firmness of strawberries displays a large natural variability, part of which can be reduced by large sample size (Doving & Måge, 2002). However, in our experiment, the contribution of sample size to the variability was limited by our careful selection of the strawberries for the experiment. A good example of this is the report from Hernández-Muñoz et al. (2008) who measured 25 strawberry fruits, but the variation of firmness was still very large. Similar variations were also found by Pelayo et al. (2003).

Decay rate of strawberry fruits was slightly slowed by the 1% CaCl$_2$ treatment during the first 10 days of storage (Fig. 1B), due to the protection of calcium against fungal contamination by reinforcing fruit tissues (Lara et al., 2004). The strawberry fruits treated with 4% CaCl$_2$ had the highest decay rate, which was possibly due to phytotoxicity by the high concentration calcium treatment. The phytotoxicity could lead to cell wall disintegration and substrate delocalization (Manganaris et al., 2007; Souty et al., 1995). The effect of CaCl$_2$ treatment on the weight loss of strawberry stored at 4°C reveals that CaCl$_2$ treatment reduced fruit weight loss, and this protective effect was especially remarkable for 4% CaCl$_2$ treatment (Fig. 1C).

There was significant difference in the contents of SSC among the groups at the beginning of the 5 days of storage (Fig. 1D). On average, fruit with 1% CaCl$_2$ treatment had significantly low SSC since 1% CaCl$_2$ may reduce the fruit respiration and metabolic activity (Mahmud, Al Eryani-Raqeeb, Syed Omar, Mohamed Zaki, & Al Eryani, 2008) resulting in less SSC than for the control group. This result was somewhat different from another report, suggesting that there was no significant difference of SSC between calcium treated and untreated strawberry fruits (Souza, Scalon, Chitarra, & Chitarra, 1999). The difference might be due to different cultivars, extended ripening process, and polysaccharide changes (Cordenunsi et al., 2003; Mahmud et al., 2008; Ohta, & Voragen, 1993). However, the 1% CaCl$_2$–treated fruits showed significantly low SSC after 5 days and 15 days of storage, which is similar to the report on ‘Lindo’ apricots (Antunes et al., 2003). While SSC of the 4% CaCl$_2$–treated fruits remained higher than those of the other groups, it seems that phytotoxicity in the 4% CaCl$_2$ group affected consumption of the substrates, which contributed to the high SSC.

During storage, pH of the fruits had a negative changing trend which was opposite to that of TA (Fig. 1E and F). While pH of the 1% CaCl$_2$ group was higher than that of the control group during the whole storage, pH was much more variable among differently treated fruits after 10 days of storage. The increment of TA at the last stage of storage was probably due to the synthesis of ascorbic acid from D-glucose, a monosaccharide normally found in strawberry fruit (Mahmud et al., 2008; Nunes, Brecht, Morais, & Sargent, 2006). The increased total acidity resulted from the increment of Cl$^-$ ions (Souty et al., 1995). The range of TA contents was comparable to that in a previous report (Souza et al., 1999).

It should be noted that the objective of this study was to investigate the relationship between the quality attributes and cell wall polysaccharides of strawberries. The degradation of cell wall polysaccharide chains was illustrated by the statistical analysis of the chain widths and lengths. We initiated the idea of using a composite sample for elucidating the cell wall degradation. Considering the variation of the individual sample, we can not draw any conclusion about the quantitative chain rules if individual fruit were analysed. For a comparative study, we used a composite sample for corresponding pH and TA analyses while SSC was determined in 20 fruits individual for each treatment.

3.2. Changes of WSP, CSP and SSP contents

The degree of fruit ripeness particularly affected the pectin contents, which supports the hypothesis that softening is closely related to pectin solubilisation and depolymerization (Kurz et al., 2008; Rosli et al., 2004). The contents of three pectins of strawberry cell wall after calcium treatments are shown in Fig. 1G–I.

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Significant relationship between two parameters at $P < 0.05$.

Significant relationship between two parameters at $P < 0.01$.

Significant relationship between two parameters at $P < 0.001$. 

Table 1: Correlation matrix of physicochemical properties and WSP, CSP and SSP contents of strawberry during cold storage.

r Firmness SSC TA pH WSP CSP SSP

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*ns* means there was no significant relationship here.
This shows that the content of SSP increased significantly, though WSP and CSP contents did not change as distinctly as did SSP during the storage time. WSP content decreased from 53.0 mg/100 g FM to 45.6, 40.3 and 33.7 mg/100 g FM in CK, 1% CaCl₂, and 4% CaCl₂ groups, respectively. CSP content slightly decreased for the first 5 days though, at the end of storage, it increased to a level similar to that of initial storage. SSP content at the end of storage was about two-fold that of the initial, which was consistent with 'Cangfangzaosheng' peaches under 2 °C cold storage (Nunes et al., 2006).

The correlations among physicochemical properties and contents of these three kinds of pectins are shown in Table 1. There was a considerably close relationship between firmness and content of CSP, revealing that the decrease of CSP content was one reason for the strawberry softening. Similar correlations were reported for bush butter fruits (Missang et al., 2001) and peaches (Brummell et al., 2004). The correlation coefficients among firmness, CSP and SSP of the control group were opposite to those of CaCl₂-treated groups, which indicates that the changes of firmness have close relationships with CSP and SSP content modifications of strawberries. The result also shows that CaCl₂ treatment affected CSP and SSP more than WSP. This was because both CSP and SSP were closely related to Ca²⁺ levels in the cell wall.

3.3. Qualitative results of nanostructures of WSP, CSP and SSP

Pectic substances hold plant cells together and their degradation would result in textural modifications, such as fruit softening (Liu et al., 2009). The degradation of pectin includes not only the content modifications, but also the nanostructural changes. Fig. 2 shows typical plane and 3-dimensional images of WSP, CSP and SSP from strawberry flesh at harvest (0 D) and the last day of storage (15 D). As shown in Fig. 2A1–E1, most of strawberry WSPs were long and single chains (notes as ‘Ls’) without branches. Though CSP was multi-branched at harvest (noted as ‘Br’ in Fig. 2A2 and B2), the branches decreased significantly during storage, as shown in Fig. 2D2, E2 and F2 compared with Fig. 2A2 and B2. Similar changes of pectin branches were found in tomatoes and apricots as well (Liu et al., 2009; Round et al., 2001).

The decrease of CSP branches during storage could be the major reason for the changes of CSP content and firmness of strawberry fruits. Since CSP was considered to be dissolved unesterified...
pectins from the cell wall middle lamella cross-linked by calcium and magnesium ions (Kurz et al., 2008), these branched structures represented branching of the pectin polygalacturonic acid backbone (Kirby et al., 2008). The side chains of CSP could be degraded during storage because the carboxyl-based crosslinking sites were destroyed due to the action of pectin-degrading enzymes, and thus, decreased branches were observed (Cosgrove, 2005). The reason why SSP chains were shorter than WSP chains, holding fewer branches than CSP, as shown in Fig. 2A3–E3, may be that SSP was comprised of the side chains of rhamnogalacturonan regions (Round et al., 2001). Overall, the visual changes of WSP and SSP during storage were not as significant as those of CSP.

3.4. Quantitative results of nanostructures of WSP, CSP and SSP

The width, length and height of pectin chains changed, accompanied by the cell wall polysaccharide degradation during fruit storage. With AFM, an appropriate instrument, the quantitative results, e.g. the width, length and height values of a specified chain, can be obtained. Table 2 and Fig. 3 show the frequency of particular length changes of WSP and SSP at harvest and the last period of storage. The detailed height results of pectin chains were not provided since they changed without any certain rule.

For length results, most of the CSP chains were multi-branched at harvest; thus the lengths of single chains could not be counted. For WSP, at harvest, frequency of length <100 nm of WSP was 11.8%, while it increased to 41.7%, 36.8% and 35.0% in the CK, 1% treatment and 4% CaCl₂ treatment groups, respectively for the last period of storage. In the meantime, WSP with long chain length (>1000 nm) decreased during storage (Table 2), which indicates that the WSP backbone degraded during fruit ripening. SSP lengths showed trends similar to WSP; while SSP of chain length <50 nm did not appear at harvest, it increased dramatically during storage. In the meantime, during storage, the frequency of SSP lengths of 51–100 nm and 301–500 nm increased significantly while the frequency of SSP length >700 nm decreased, which indicates degradation of SSP (in length) during storage.

In general, the average lengths of WSP and SSP at harvest were 837.17 nm and 671.17 nm, respectively. At the final stage of storage, the average lengths were 585.44 nm, 591.98 nm and 587.69 nm for WSP and 421.31 nm, 424.66 nm and 406.51 nm for SSP in the CK group, 1% CaCl₂ treatment and 4% CaCl₂ treatment groups, respectively. The lengths were reduced by 30.07%, 29.29% and 29.80% for WSP and 37.23%, 36.73% and 39.43% for SSP, respectively, suggesting that the main chains of SSP were degraded more significantly than were those of WSP in strawberry during storage.

Fig. 2 (continued)
Compared with the CK group, CaCl₂ treatment did not affect the changes of WSP chain lengths distinctly after 15 d of strawberry storage, considering that there were no significant differences of Fq of chains with length <100 nm and length >700 nm between CK group and CaCl₂ treatment groups at the end of storage. However, CaCl₂ treatment delayed the degradation of SSP main chains. The Fq of SSP chains with length <50 nm was less and Fq of SSP chains with length >700 nm was more in the CaCl₂ treatment groups than the CK group. CaCl₂ treatment increased the content of Ca²⁺ in the cell wall, strengthening the network structure of SSP and inhibiting the degradation of main chains of these pectins.

Table 3 shows the three pectin width changes of differently treated strawberries during storage. In general, the width of SSP chains was smaller than those of WSP and CSP. This may be partially due to β-elimination during extraction, caused by sodium carbonate (Kirby et al., 2008). In addition, SSP was richer in neutral sugars, and was comprised of side chains of rhamnogalacturonan regions, specifically arabinose and galactose (Round et al., 2001). Neutral sugars, coiled or aligned along and around the rhamnogalacturonan regions, presented as short branches, likely caused changes in the width of pectin chains (Round et al., 2001).

The width changes of pectin chains reflected the degradation of pectin short branches. The widths of WSP, CSP and SSP, measured by AFM, all decreased after storage (Table 3). For instance, Fq of smaller width 23.438 nm of WSP at harvest was 10.1%; the values were 22.7%, 17.4% and 27.3% for CK, 1% CaCl₂ treatment, and 4% CaCl₂ treatment groups, respectively on day 15. Fq of 23.438 nm of CSP at harvest was 5.6%; the values were 11.8%, 7.7% and 7.1% for the three groups, respectively on day 15. Fq of widths of both 15.594 nm and 19.531 nm for SSP increased from 0 to more than 20% for all the groups. Meanwhile, the Fq of larger width chains declined. Taking SSP for instance, the Fq of widths from 46.875 nm to 78.125 nm was 50% (12.5% + 37.5% + 0%) for strawberry at harvest; the values were 14.3%, 19.1% and 15% for CK, 1% CaCl₂ treatment and 4% CaCl₂ treatment groups, respectively on day 15. This result also shows that the Fq of large width in the CK group decreased faster than those of the CaCl₂ treatment groups. The trend was similar to the results of peaches (Yang et al., 2006) and apricots (Liu et al., 2009). All these results suggest that the side chains of pectins were degraded during cold storage of those fruits.

The modifications of width of CSP and SSP during storage were more obvious than those of WSP, especially in the CK group, as shown in Table 3. For instance, the Fq of WSP chains with large widths of 58.594 nm and 78.125 nm, in the CK group, did not decrease as remarkably as those of CSP and SSP. CSP was considered to be cross-linked by calcium mainly in the cell wall middle lamella. SSP became free by Na₂CO₃ extraction, which broke ester linkages mainly from the primary cell wall (Round et al., 2001). As these two pectins, CSP and SSP, bear close relationships with fruit firmness (Brummell et al., 2004), the degradation of side branches of CSP and SSP during storage might be the main reason for strawberry softening.
Table 2
Length changes of WSP and SSP chains from strawberry at harvest (0 D) and on the 15th (15 D) day of storage after calcium treatment.

<table>
<thead>
<tr>
<th>L (nm)</th>
<th>0 D</th>
<th>15 D-CK</th>
<th>15 D-1% CaCl₂</th>
<th>15 D-4% CaCl₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T(Fq%) Ave (nm)</td>
<td>T(Fq%) Ave (nm)</td>
<td>T(Fq%) Ave (nm)</td>
<td>T(Fq%) Ave (nm)</td>
</tr>
<tr>
<td>WSP</td>
<td>101–300</td>
<td>2(11.8) 88.2 ± 10.3</td>
<td>5(41.7) 90.3 ± 9.4</td>
<td>7(36.8) 87.6 ± 10.2</td>
</tr>
<tr>
<td></td>
<td>&gt;2000</td>
<td>1(5.9) 3043.1 ± 0</td>
<td>0(0) –</td>
<td>0(0) –</td>
</tr>
<tr>
<td></td>
<td>Ave (nm)</td>
<td>837.17</td>
<td>585.44</td>
<td>591.98</td>
</tr>
<tr>
<td>SSP</td>
<td>&lt;50</td>
<td>0(0) –</td>
<td>5(25.0) 43.2 ± 12.7</td>
<td>4(18.2) 32.5 ± 9.4</td>
</tr>
<tr>
<td></td>
<td>51–100</td>
<td>3(12.5) 87.2 ± 3.4</td>
<td>4(20.0) 67.3 ± 35.4</td>
<td>5(22.7) 79.3 ± 12.7</td>
</tr>
<tr>
<td></td>
<td>101–300</td>
<td>3(12.5) 245.9 ± 124.8</td>
<td>5(25.0) 239.1 ± 77.9</td>
<td>3(13.6) 257.8 ± 37.5</td>
</tr>
<tr>
<td></td>
<td>&gt;2000</td>
<td>1(5.9) 3043.1 ± 0</td>
<td>0(0) –</td>
<td>0(0) –</td>
</tr>
<tr>
<td></td>
<td>Ave (nm)</td>
<td>671.17</td>
<td>424.66</td>
<td>406.51</td>
</tr>
</tbody>
</table>

Note: 'L' means the lengths of WSP and SSP chains. 'T(Fq%)' means the times and frequency of length value in a particular range. 'av' means the average value of several chains in the particular range and 'Ave' means the total average value of all WSP and SSP lengths of each group.

Fig. 3. Length distribution changes of WSP and SSP chains from strawberries at harvest (0 D) and on the 15th (15 D) day of storage after CaCl₂ treatment.

Table 3
Width changes of WSP, CSP and SSP chains from strawberry at harvest (0 D) and on the 15th (15 D) day of storage after calcium treatment.

<table>
<thead>
<tr>
<th>W (nm)</th>
<th>0 D</th>
<th>15 D-CK</th>
<th>15 D-1% CaCl₂</th>
<th>15 D-4% CaCl₂</th>
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<tbody>
<tr>
<td></td>
<td>CK</td>
<td>1% CaCl₂</td>
<td>4% CaCl₂</td>
<td>CK</td>
</tr>
<tr>
<td>15.594</td>
<td>0(0)</td>
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<td>0(0)</td>
<td>0(0)</td>
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<tr>
<td>19.531</td>
<td>0(0)</td>
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<td>58.594</td>
<td>0(0)</td>
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<tr>
<td>78.125</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
</tbody>
</table>

Note: ‘W’ means the widths of WSP, CSP and SSP chains. ‘T(Fq%)’ means the times and frequency of particular width value at harvest (0 D) and at last storage period (15 D).
CaCl$_2$ treatment did not prevent WSP side chains from significant degradation, as shown in Table 3, which was consistent with the subtle effect of CaCl$_2$ treatment on the content of WSP. Fq of CSP chains with small value widths was smaller in the CaCl$_2$ treatment group than in the control group. For small widths of 23.438 nm, the Fq was 11.8% in the CK group, 7.7% in the 1% CaCl$_2$ treatment group and 7.1% in the 4% CaCl$_2$ treatment group. Width of the SSP showed a similar trend, indicating that CaCl$_2$ treatment inhibited degradation of large widths of CSP and SSP chains toward small widths during storage. The effect of calcium was due to homogalacturonans, ionically crosslinked by calcium between pectin molecules, and CaCl$_2$ treatment increased the content of Ca$^{2+}$, leading to strengthened crosslinking between pectins (Liu et al., 2009). CaCl$_2$ treatment decreased the degradation of CSP and SSP at the nanostructural level, which was in accordance with content changes, and which might be the main reason for CaCl$_2$ effects on physicochemical modifications of strawberry fruit during cold storage.

These AFM qualitative images and quantitative results provided direct morphological evidence of pectin degradation. In Fig. 4, a schematic model to show the degradation of strawberry pectins during storage is proposed. In the model, the branches, width and length values of pectin chains were significantly reduced at the last stage of storage. CaCl$_2$ treatment delayed the chain degradation.

In conclusion, CaCl$_2$ treatment delayed the changes of physicochemical properties, as well as the degradation of three kinds of pectins, of strawberry fruits, by strengthening the ionic crosslinkages among these pectin molecules. Modifications of CSP and SSP, especially the side chains, resulted in strawberry softening. A schematic model was proposed for demonstrating the role of CaCl$_2$ treatment in the degradation of pectins. The results suggest that CaCl$_2$ extends the shelf life of postharvest strawberries by influencing the degradation of pectin molecules.

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**References**


