



Chitosan combined with calcium chloride impacts fresh-cut honeydew melon by stabilising nanostructures of sodium-carbonate-soluble pectin

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ABSTRACT

Effects of chitosan, calcium chloride, and their combination on preventing quality and safety deterioration of postharvest fresh-cut honeydew melon at 7 °C were investigated. Treatments of all these three delayed weight loss, improved firmness, delayed colour changes and moderately inhibited microbial growth. Interestingly, combined treatment of chitosan and calcium chloride was the most effective, leading to 40% less weight loss, 45% (3.70 N) increase in firmness, less overall colour difference, and more than 0.5 log CFU/g inhibition on mesophilic and psychrotrophic growths on day 13 of storage as compared to control group. In addition, these treatments also inhibited degradation of pectin chains. Nanostructural analysis of fruit pectin via atomic force microscopy (AFM) indicated that firmness was closely related to the nanostructure of sodium carbonate-soluble pectin (SSP). Short and narrow SSP chains were observed more in samples with less firmness. We conclude that edible coating of chitosan combined with calcium chloride treatment synergistically extends the shelf-life of fresh-cut honeydew melon by maintaining the integrity of SSP via interactions between SSP and calcium ions or protonated chitosan groups.

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1. Introduction

Fresh-cut fruits refer to fruits that have undergone process of alteration from its original state. They are increasingly demanded by consumers due to convenience, especially for those having busy lifestyle. However, fresh-cut fruits are highly perishable and deteriorate much faster than the whole counterpart due to the exposure of inner flesh to environment, thus they have much shorter shelf-life (Mantilla, Castell-Perez, Gomes, & Moreira, 2013) and request better procedure to protect their quality and safety. Honeydew melon (*Cucumis melo* L. var *inodorus*) is a climacteric, favourable fruit to consumers. It is very common to be marketed in fresh-cut state due to convenience (Amaro, Beaulieu, Grimm, Stein, & Almeida, 2012). However, once the fruits deteriorate to a certain

extent, significant texture breakdown and colour changes occur which lead to appearance changes followed by the end of shelf-life.

Currently, one approach for extending the shelf-life of fresh-cut fruits is applying edible coating on the fruit surface. Edible coating potentially prolongs the shelf-life of fruits due to the formation of barrier layer blocking the exposure of gas and moisture. The barrier layer can delay the quality deterioration of fruits by reducing the respiration rate, delaying volatiles loss, decreasing the rate of moisture migration as well as other quality deterioration processes and thus extend the shelf-life of fruits (Rojas-Grau, Soliva-Fortuny, & Martin-Belloso, 2009).

Texture is an important factor to determine the organoleptic properties of fruits. Texture or the rate of tissue softening is closely related to depolymerisation and solubility of cell wall polysaccharides thus shelf-life during postharvest storage of fruits and vegetables (Liu et al., 2009). To protect texture properties of post-harvest produce, a technique that has been shown effective is applying calcium salt, which has been shown to enhance the textural properties of fruits such as strawberries and apples (Lara, Garcia, & Vendrell, 2004; Liu et al., 2009; Qi, Hu, Jiang, Tian, & Li,

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2011; Silveira, Aguayo, Chisari, & Artés, 2011; de Souza, O'Hare, Durigan, & de Souza, 2006; Wang, Xie, & Long, 2014). Another approach is edible coating like chitosan. It has been applied successfully as coating on food surfaces to extend the shelf-life effectively without compromising the natural taste of foods (Chien, Sheu, & Yang, 2007; Fernández-Saiz, Sánchez, Soler, Lagaron, & Ocio, 2013; Wang, Wu, Qin, & Meng, 2014; Xiao et al., 2010). Due to its antimicrobial property, chitosan coating is very effective against spoilage for fruits as indicated by evidence from fresh-cut pears and fresh-cut papayas (Brasil, Gomes, Puerta-Gomez, Castell-Perez, & Moreira, 2012; Xiao, Luo, Luo, & Wang, 2011). Though these two approaches are effective for many postharvest fruits, the in-depth knowledge of the effective site of the fruits is largely unknown.

Apart from parameters of physicochemical properties that are indicators of fruit quality directly, atomic force microscopy (AFM), a powerful nanotechnology tool for examining food structure and molecule at nanostructure level is useful in obtaining qualitative and quantitative information of produce quality and safety. It has been applied on characterising nanostructure of fruit pectins water-soluble pectin (WSP), chelate-soluble pectin (CSP) and sodium carbonate-soluble pectin (SSP) (Chen et al., 2011; Liu et al., 2009; Zhang et al., 2008).

The objective of this work was to elucidate the fundamental effects of chitosan coating and calcium chloride coating on inhibiting postharvest quality deterioration, mainly via nanostructural analysis. The work evaluated the physicochemical properties of postharvest honeydew melon, antimicrobial property, and nanostructures of fruit pectins under coating circumstances.

2. Materials and methods

2.1. Fruit materials and coating solution preparation

Chitosan coating was prepared according to a method described by Hernandez-Munoz, Almenar, Del Valle, Velez, and Gavara (2008) with slight modification. The powder (20 g) bought from Zhengzhou Yanlord Chemical Products Co., Ltd (Zhengzhou, Henan, China) was dissolved in 1 L of distilled water and homogenized by magnetic stirrer. Glycerol (1.5%, w/v) was added into the mixture as a plasticiser.

Honeydew melon (*Cucumis melo* L. var *inodorus*) imported from Malaysia was purchased from a local Singapore supermarket. Fruits were chosen based on similarity in colour, size, ripening stage, and absence of visible defects. All fruits were stored at 7 °C prior to experiments. The honeydew was washed with sterile deionized water, peeled and cut into cubes (2 cm × 2 cm × 2 cm) (Chien et al., 2007). The fruit samples were randomly distributed for being subject to the treatments as mentioned below: distilled water as control group; 2% chitosan; 1% CaCl₂; 1% CaCl₂ + 2% chitosan (w/w). Fruit samples were coated by dipping in the coating solutions for 10 min followed by 1 h drying in air. Then all samples were kept in Ziploc bags (6 cm × 8 cm, thickness = 0.05 mm) and stored in a refrigerator (7 ± 2 °C) up to 13 days.

2.2. Determination of weight loss, firmness, soluble solids content (SSC), pH value and titratable acidity (TA)

For weight loss, 5 individual fruits each group at each sampling time point was taken out from cold storage, weighed after 3 h and calculated using the formulae: Weight loss (%) = $(m - m_0)/m_0$, in which m and m_0 indicate the individual weight at present and initially, respectively (Chen et al., 2011). Firmness of samples was determined by TA-XT2i Texture analyser (Stable Micro Systems Ltd, Godalming, UK). A ball probe with diameter of 5 mm was used and

test condition was set according to Benítez, Achaerandio, Sepulcre, and Pujolà (2013) with slight modification: test speed of 1 mm/s, penetrating distance of 5 mm and trigger force of 5 g. Firmness of samples was expressed as peak force (N), which was the maximum resistance force experienced by samples in the puncture test and obtained from the force–distance curves. These tests were conducted with five replicates for each group.

SSC was measured on fruit juice extracted from 50 g sample using a digital refractometer (Nova-Tech International, Inc., Houston, TX, USA) and expressed as °Brix. pH value was measured by a pH meter (Metrohm Singapore Pte, Ltd, Singapore). For TA, 10 ml juice was mixed with 10 ml distilled water and 5 ml diluted juice was titrated against 0.1 M of sodium hydroxide after addition of indicators, phenolphthalein. TA is expressed as percent malic acid (Chen et al., 2011).

2.3. Colour analysis

Minolta Colorimeter CM-3500d (Konica Minolta, Inc., Japan) was used for colour analysis of sample surface. The colour coordinates, values of L , a , b , were determined from the colorimetry system and overall colour difference was calculated by applying formula as described by Geraldine, Soares, Botrel, and Goncalves (2008),

$$\Delta E^* = \sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]}$$

where ΔE^* represents the overall colour difference. Standard white plate and black plate were used for instrument calibration.

2.4. Microbiological analysis

Each sample (25 g) was put in a sterile stomacher bag, homogenised with sterile peptone water (0.1%, 225 ml) and serial dilutions of samples were prepared. Each sample (0.1 ml) was used for spread plating. Aerobic mesophilic count was enumerated from standard plate count agar (PCA), which was incubated at 37 °C for 2 days. Aerobic psychotropic count was enumerated from PCA with 10 days incubation at 7 °C. Yeasts and moulds were counted based on potato dextrose agar (PDA) with incubation at 25 °C for 4 days. The results were expressed as log CFU/g sample (Benítez et al., 2013; Chien et al., 2007).

2.5. Pectin extraction and analysis

2.5.1. Cell wall preparation and pectin extraction

Cell wall materials (before treatment, day 1, 7, 13) were extracted according to method described by Chen et al. (2011) with slight modification. Samples (10 g) were ground and boiled in 200 ml ethanol (80%, v/v) for 20 min. After cooled to room temperature, samples were filtered and the extraction process was repeated two more times. Residue was incubated in 50 ml mixture of dimethyl sulphoxide (DMSO) and water (9:1, v/v) at 4 °C overnight. Then the residue was dipped in 200 ml mixture of chloroform and ethanol (2:1, v/v) for 10 min and washed with acetone until total whitening. The residue was collected as cell wall materials.

Distilled water (10 ml) was added into cell wall materials and agitated for 4 h at 25 °C for WSP extraction. Supernatant was collected after centrifugation at 10,000 g 4 °C for 10 min. The above process was repeated twice before addition of 10 ml 50 mM CDTA (Sigma–Aldrich) for CSP extraction. After the extraction process was repeated twice more, supernatants were combined together and collected as CSP. SSP was obtained by further extraction of the residue with 10 ml 50 mM sodium carbonate (Sigma–Aldrich)

containing 2 mM CDTA. The extraction was repeated twice more followed by centrifugation.

2.5.2. Determination of pectin contents

The extracted pectin content was determined by Carbazole colorimetry and galacturonic acid (Sigma–Aldrich, MO, USA) was used as a standard. Sulphuric acid (98%, v/v) (12 ml) was added into pectin sample (2 ml) and cooled by ice bath. The mixture was boiled for 10 min and cooled by tap water. It was then incubated at room temperature for 30 min and added 0.5 ml carbazole-ethanol solution (0.1%, w/v) (Sigma–Aldrich). Absorbance at 530 nm was measured by a UV-2000 spectrophotometer (Spectra-Teknik (S) Pte, Ltd, Singapore) and the pectin content was expressed as mg galacturonic acid/100 g fresh weight (FW) (Chen et al., 2011; Liu et al., 2009).

2.5.3. Nanostructural characterisation of SSP

Atomic force microscopy (TT-AFM, AFM workshop, Signal Hill, CA, USA) was used to perform nanostructural analysis of pectin. Sample was subject to appropriate dilution and 10 μ l of sample was pipetted on a freshly cleaved mica sheets and dried by using a rubber suction ball. The imaging condition was set as vibrating mode, scan rate of 0.4 Hz and scan lines of 128–512. NSC 11/no Al tips with resonance of 145–240 kHz and force constant of 25–95 N/m were used (Chen et al., 2013; Yang, Chen, An, & Lai, 2009). AFM images were analysed offline by using Gwyddion software (AFM workshop). Qualitative information was obtained through identification of pectin characteristics from AFM images, such as branch chains, aggregates and others. Quantitative data were obtained by sectional analysis on length, height and width of pectin chains from images using the software (Xin et al., 2010).

2.6. Statistical analysis

Results were reported as means \pm standard deviations. Analysis of variance (ANOVA) and Duncan's test were performed to examine the differences between groups by SAS software (SAS Institute Inc., Cary, NC, USA). Comparisons with *P* value less than 0.05 were considered statistically significant.

3. Results and discussion

3.1. Weight loss, firmness, SSC, pH and TA

Fig. 1 shows the effects of different treatments, 2% chitosan, 1% calcium chloride, and control groups on weight loss, firmness, SSC, pH and TA of honeydew melon. The loss of fruit weight during postharvest storage can be approximately attributed to the loss of water because fruits contained a large amount of water which is relatively easy to evaporate (Vasey, 2006). As shown in Fig. 1a, weight loss for all groups increased with storage time. Interestingly weight loss of control group was significantly higher ($P < 0.05$) than all the other groups. More specifically, weight loss of all treated groups was less than 1% at day 7 and reached 1.5% at day 10 while weight loss of control samples was around 2% at day 7 and approximately 2.5% and 3.5% at day 10 and day 13, respectively. These data indicate that the treatments can effectively prevent moisture loss of honeydew melon by coating fruit surface, the migration of moisture from inner of fruits to surface slowed down, leading to reduced rate of moisture loss and possibly reduced respiration rate (Qi et al., 2011; Xiao et al., 2010). Our results were consistent with other reports (Chen et al., 2011; Chien et al. 2007). In addition, combined chitosan and calcium had the best protective effect on weight loss, indicating that they may have synergistic effect on retarding weight loss of

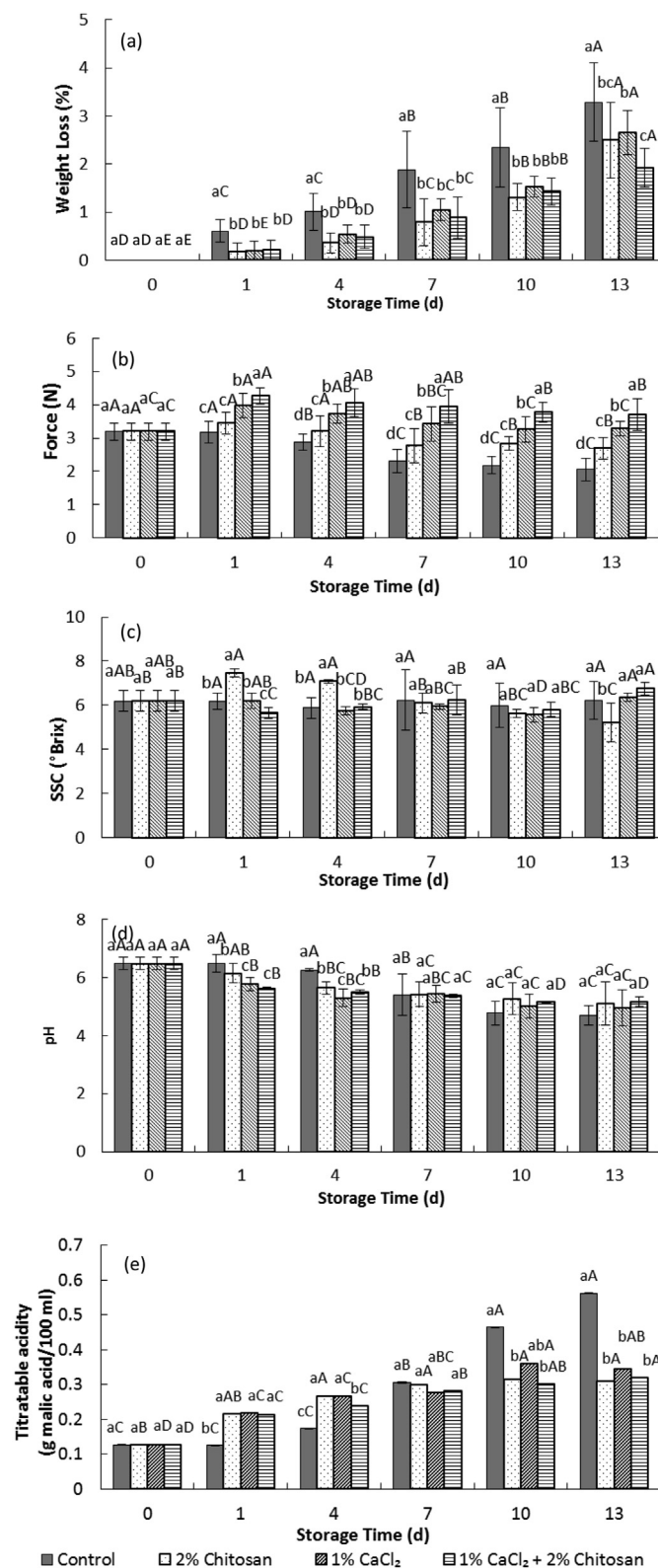


Fig. 1. Effect of different treatments on weight loss, firmness, soluble solid contents (SSC), pH and titratable acidity (TA) of honeydew melon. (a) weight loss; (b) firmness; (c) SSC; (d) pH; (e) TA of honeydew. Data are presented as means \pm standard deviation. Means within same storage time with different small case letters are significantly different; means for same treatments on different storage times with different capital letters are significantly different.

honeydew melon. This result agrees well with a study on fresh-cut apple treated with combination of 0.5% CaCl₂, 2% ascorbic acid and 1% chitosan coating (Qi et al., 2011).

Firmness is one of the important parameters reflecting the postharvest storage characteristic of fruits. It is related to tissue softening. Loss of firmness caused by the loss of integrity of cell wall structure leads to wilting and shrinking of fruits (Qi et al., 2011). As shown in Fig. 1b, firmness of the initial samples was 3.19 N before any treatments. After 1 day of coating processing, firmness of calcium treated groups was significantly ($P < 0.05$) higher than untreated control group. With calcium salt treatment, the firmness of fruit increased slightly in the first couple of days of storage; this might be due to chelate effect of calcium ions on pectin and other related macromolecules within fruit flesh, leading to increased firmness. This finding is consistent with previous reports (Ngamchuachit, Sivertsen, Mitcham, & Barrett, 2014; Saftner, Bai, Abbott, & Lee, 2003; Varela, Salvador, & Fiszman, 2007). The concentration of coating solution was very low (<3%) in all the groups. After treatment and drying, the coating layer was very thin, thus the firmness determined can be viewed as fruit itself though theoretically it might be partially contributed by the coating layer. This contribution to firmness by coating layer was not counted by previous reports of similar research as well (Ngamchuachit et al., 2014; Saftner et al., 2003; Varela et al., 2007). During day 1 to day 13, storage, firmness of all samples decreased. In addition, the rates of tissue softening were significantly different ($P < 0.05$) between control and all treated group. The highest firmness was observed in combined chitosan and calcium chloride treated samples, of which the firmness was within the range of 4.26 N–3.70 N in 13 days of storage, followed by calcium only treated samples and chitosan only coated samples. The current results were in agreement with previous reports that chitosan coating and calcium dipping are effective in preserving the firmness of fruits (Hernandez-Munoz et al., 2008; Liu et al., 2009; Qi et al., 2011; Xiao et al., 2010).

For SSC, there were no significant changes ($P > 0.05$) during 13 days of storage for all of the samples. As shown in Fig. 1c, decreasing trend of SSC was observed on chitosan coated samples with ranging from 7.45 °Brix to 5.21 °Brix from day 1 to day 13. On contrast, no consistent trend of °Brix was observed in calcium treated samples (between 5.57 and 6.36) and slightly increasing trend was found in combined chitosan and calcium treated samples (increased from 5.66 to 6.77) during storage period. However, for control sample, SSC was stable during 13 days of storage (between 6.19 and 5.88). For SSC at specific storage time (day 3, day 7, for instance), no significant changes were found between control sample and calcium or combined treatment groups except chitosan coated group. Thus, except chitosan coating, other coating and dipping processes did not cause significant changes on SSC of honeydew melon.

Apart from SSC, pH of all the honeydew samples was decreased throughout the storage period (Fig. 1d). All of the treated samples had slightly lower pH compared to control sample at day 1 and day 4 of storage. After day 4, no significant difference of pH was found between control and treated samples. The difference of pH at day 1 and day 4 could be partially due to the pH difference of coating solutions (deionized water: pH 7.00; 2% chitosan: pH 5.07; 1% CaCl₂: pH 6.06; 1% CaCl₂ + 2% chitosan: pH 4.32). According to Hodges (2003), pH of postharvest produce can be affected by oxidative stress, environmental stress and harvest stress (e.g., weight loss), which results in disruption of membrane integrity as well as cellular activities. The significant decrease in pH observed in control group could be largely due to these stresses which had less effect on treated samples (Hodges, 2003). These stresses also caused a reduction of ATPase pumping capacity, thus decreasing the cellular pH. However, TA was significantly increased in control

sample during 13 days of storage while it was only slightly increased in all other treated groups, as shown in Fig. 1e. TA is an indicator of pH, generally higher TA, lower pH, vice versa. These results were not consistent with reports of other fruits (Atress, El-Mogy, Aboul-Anean, & Alsanious, 2010). Determination of TA is actually assaying the amount of available organic acids within fruits. According to Liu et al. (2009), increased level of TA can be caused by increased respiration rate and production of high level CO₂, which affected the glycolytic enzyme systems and resulted in accumulation of acids. The slightly lower TA observed in treated samples as compared to control sample was suggested to be due to the coating layer acting as a protection against spoilage microorganisms, which resulted in less off-flavours of fruits and less amount of acidic compounds produced within fruit samples (Rojas-Grau et al., 2009).

3.2. Colour

Colour is one of the critical factors determining consumer acceptance on the quality of fruits because it is an indicator of the degree of freshness, extent of fruit deterioration, even infestation of disease as well as contamination (Chen et al., 2011). As shown in Fig. 2, colour was significantly different between control and all treated groups. In control group, lightness decreased from 62.75 to 46.04, *a* value increased from –1.16 to –0.26 and *b* value decreased from 13.88 to 10.44 during storage, indicating significant browning process during storage occurred which caused the darkening and loss of fresh yellow colour of fruits. Treatment effectively delayed this postharvest browning, indicated by the similar lightness (between 62.75 and 61.85) from day 1 until day 10, and slightly decreased lightness from day 10 to day 13, as well as slightly increased *a* value and similar *b* value throughout the storage period. Besides, colour changes among treatments did not vary significantly within 13 days of storage, indicating similar effectiveness of the three treatments on delaying colour changes of postharvest honeydew melon.

As shown in Fig. 2d, the overall colour difference at day 13 was significantly different between control group and treatment groups; however, there was no significant difference among the three treatment groups. The similar efficacy of chitosan coating and calcium treatment on preserving fruit colour was also reported previously in other fruits (Benítez et al., 2013; Chien et al., 2007; Hernández-Muñoz, Almenar, Ocio, & Gavara, 2006; Qi et al., 2011). The significantly less overall colour changes observed in all treated groups was highly due to the gas barrier properties of coating layers, which reduced the rate of oxygen diffusion into the fruits, resulting in less metabolic or other biochemical processes that could have caused undesirable colour changes for honeydew (Qi et al., 2011).

It should be noted that coating of high concentration calcium chloride introduced undesirable bitterness to fresh-cut fruits. Previous reports indicated that 2.5% CaCl₂ tasted bitter while 1.0% CaCl₂ was not associated with any specific taste effects (Saftner et al., 2003; Varela et al., 2007). Coating with around 2% chitosan did not have any sensory effects (Xiao et al., 2010).

3.3. Microbiological analyses

Fresh-cut fruits have a high deterioration rate due to no protection of outlayer peel. Upon removal of epidermis during postharvest minimal processing, the physical and chemical barriers against spoilage microorganisms, yeasts and moulds were lost, resulting in increased decaying rate and greatly shortened shelf-life of fruits. Coating with antimicrobial agents could retard the microbial growth. According to Rojas-Grau et al. (2009), microbial

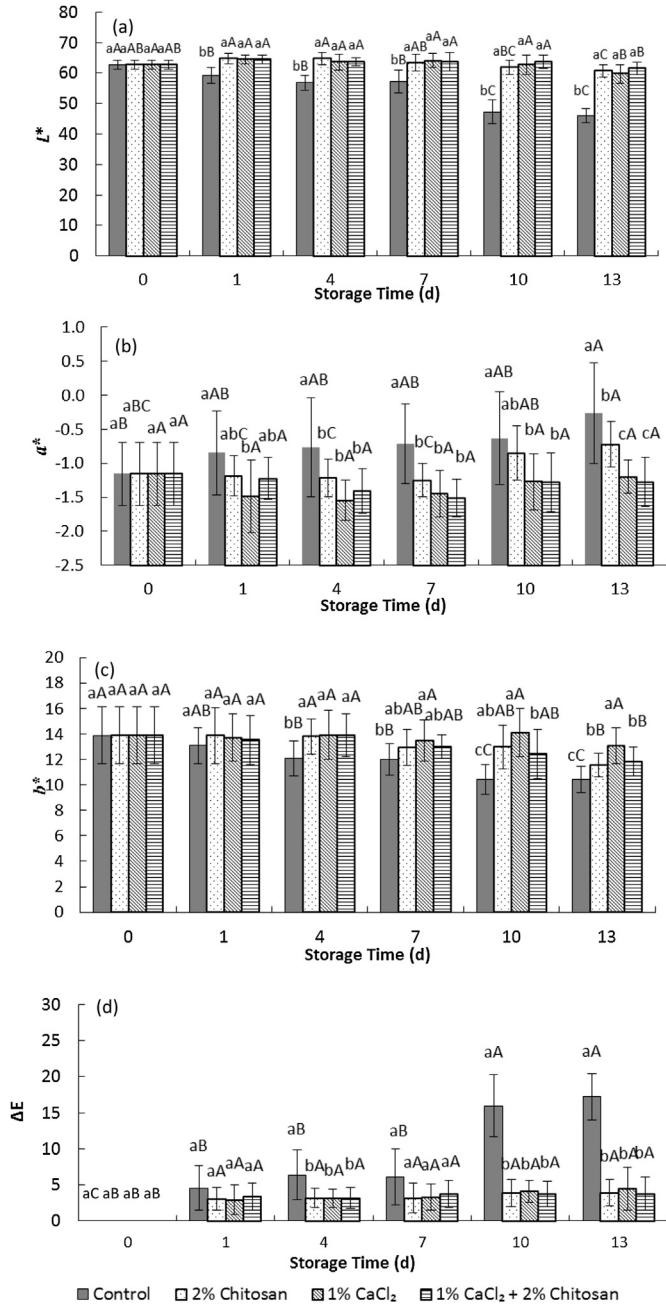


Fig. 2. Effect of different treatments on colour changes of honeydew melon. (a) L^* ; (b) a^* ; (c) b^* ; (d) overall colour difference of honeydew. Data are presented as means \pm standard deviation. Means within same storage time with different small case letters are significantly different; means for same treatments on different storage times with different capital letters are significantly different.

survivals can be effectively controlled by application of coating containing antimicrobial agents on fresh-cut fruits, thus the shelf-life of fruits extended.

As shown in Fig. 3a–c, total viable counts of mesophilic bacteria (3.45–5.83 log CFU/g), psychrotrophs (3.86–6.57 log CFU/g), yeasts and moulds (3.56–6.65 log CFU/g) in control groups were significantly higher than treated samples from storage of day 1 to day 7. Approximately 0.5–1 log reduction of total viable counts was observed in treated groups compared to control group. In addition, the total viable counts of mesophilic bacteria did not vary significantly among treatment groups during 13 days of storage.

Moreover, significant reductions in psychrotroph populations were found in combined chitosan and calcium chloride treatment group compared to the other two chitosan only and calcium chloride only treatment groups. The antimicrobial effect of coating was reports on other fruits as well (Chen et al., 2011; Chien et al., 2007; Tezotto-Uliana, Fargoni, Geerdink, & Kluge, 2014). From these results, calcium chloride is more effective in controlling yeasts and moulds than chitosan (Fig. 3b). In some groups, count of psychrotrophic was higher than that of mesophilic aerobic bacteria (Fig. 3a and b), though generally the latter is higher. This result was supported by report on some fruits (Serradilla et al., 2013). Though the aerobic mesophilic counts at days 7 and 13 were pretty high, they were comparable to previous reports of marketing fruits in Singapore and USA (Seow, Ágoston, Phua, & Yuk, 2012; Valentin-Bon, Jacobson, Monday, & Feng, 2008), indicating that microbial counts need to be controlled for fresh-cut fruits.

Chitosan has unique functional properties attributed to the chemical structure of protonated amino groups that confer an ability to interact with negative residues (i.e. phosphoryl groups)

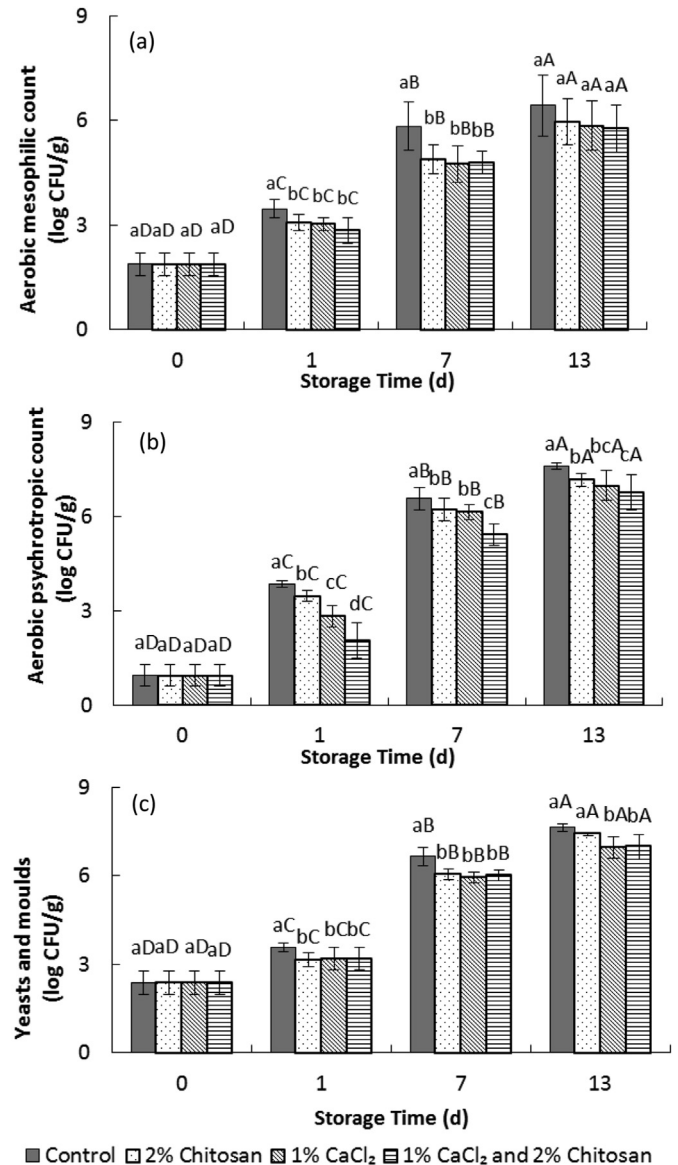


Fig. 3. Effect of different treatments on microbial survivals of honeydew melon (a) aerobic mesophilic count; (b) aerobic psychrotrophic count; (c) yeasts and moulds.

present on the surface of microbial cells (Tezotto-Uliana et al., 2014). The electrostatic interaction between chitosan and microbial cells either caused the alteration of microorganism's membrane permeability, leading to disruption of osmotic balance and leakage of intracellular electrolytes, followed by microbial growth inhibition (Goy, Britto, & Assis, 2009), or resulted in microbial growth suppression by acting on microbial DNA and affecting the synthesis of mRNA and protein (Goy et al., 2009).

CaCl₂ can prevent fruits postharvest decay through strengthening and reinforcing cell wall structure, thus providing a protective barrier against microbes for fruits (Chen et al., 2011). CaCl₂ also decreased postharvest fruit decaying by lowering fruit intracellular pH as well as reducing its water activity, resulting in bacteriostatic action and inhibitory effect on microbe growth (Atress et al., 2010).

The initial treatment on the fresh-cut honeydew melon in the current research only involved deionized water treatment but not chlorine sanitiser treatment as reported by many other groups (Ngamchuachit et al., 2014; Saftner et al., 2003; Varela et al., 2007). Therefore the microbial level was quite high (>6 log CFU/g for aerobic mesophilic count, aerobic psychrotrophic count, yeasts and moulds) at day 13. We did not test the properties of the fresh-cut fruit after 13 days of storage because the microbial level was so high that the product had not sufficient marketing value.

3.4. Pectin content

Pectin is one of the major components of cell wall materials, closely related to fruit tissue softening. Because of pectin solubilisation and depolymerisation during ripening, fruit tissue softened (Kurz, Carle, & Schieber, 2008; Rosli, Civello, & Martínez, 2004). As shown in Fig. 4a, WSP content of all samples decreased from day 0 (before treatment) to day 13, with 27.56 mg/100 g FW (day 0) to 7.92, 9.77, 9.92, and 9.47 mg/100 g FW in control, 2% chitosan, 1% CaCl₂, and combined treatment groups, respectively (day 13). WSP content of control group was significantly higher than combined treatment group only on day 1 of storage. After day 1, no significant difference among all the groups was observed. For CSP, there was an increasing trend in all treated samples although in control group it was quite consistent during day 1 to day 13 storage period (Fig. 4b). Interestingly, significant difference in CSP content between control and treated samples was found at day 13. For SSP, more variations were found in control group than treatment groups. SSP amount was significantly higher in treatment groups than control group at day 7 and day 13 (Fig. 4c).

After the treatments with chitosan that contains protonated amino groups and CaCl₂ treatment that contains calcium ions, increased CSP content was observed in all treated samples. The weakly bound uronic acid (water soluble) cross-links with each other and the uronic acid from SSP solubilises to CSP fraction. All these contributed to the increased amount of ion-bounded pectins after treatment (Liu et al., 2009). However, according to Zhang et al. (2008), SSP is positively and closely related to firmness as compared to WSP and CSP. This is in agreement with the current experimental results on firmness and SSP content. For control samples, reduced firmness was corresponded with decreased SSP content over storage period, while in treated honeydews, SSP contents were maintained at high level, suggesting an improved firmness.

3.5. AFM analysis

3.5.1. Qualitative results

The changes of cell wall structures are most correlated with the textural breakdown of fruits. Degradation of cell wall polysaccharides especially pectin components contributes to these

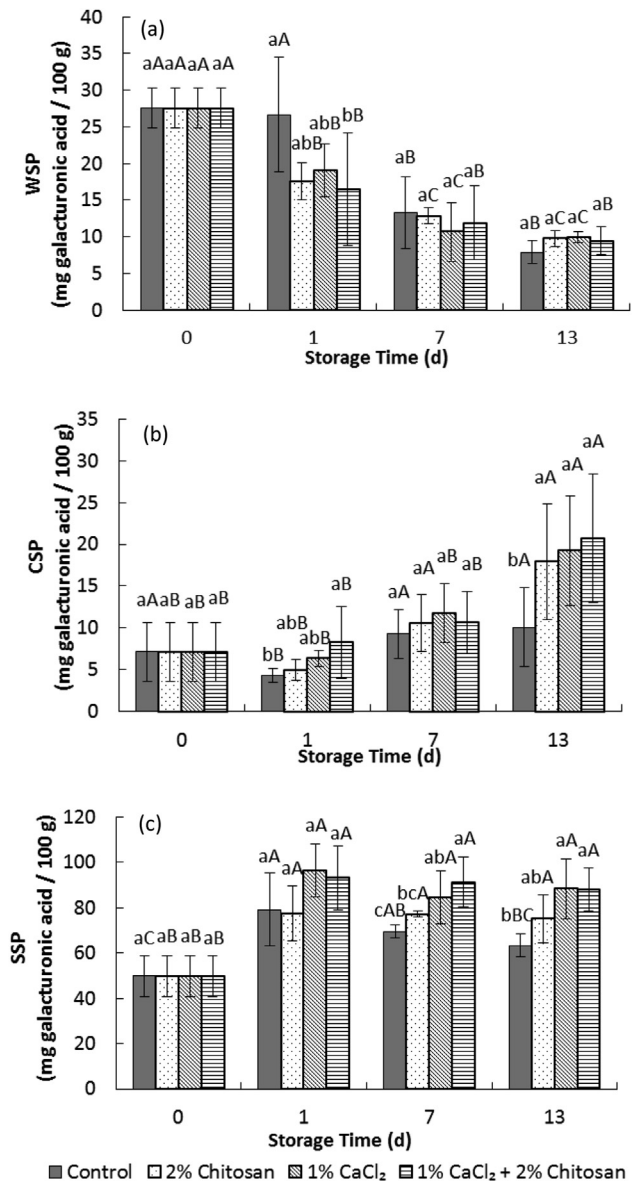


Fig. 4. Effect of different treatments on pectin contents of honeydew melon. (a) water-soluble pectin (WSP); (b) chelate-soluble pectin (CSP); (c) Sodium carbonate-soluble pectin (SSP). Data are presented as means \pm standard deviation. Means within same storage time with different small case letters are significantly different; means for same treatments on different storage times with different capital letters are significantly different.

textural changes. Pectin degradation is not only attributed to modification of pectin content, but also significantly caused by the alteration of pectin nanostructures (Chen et al., 2011; Xin et al., 2010).

Fig. 5 shows the nanostructural morphologies of pectin chains including qualitative characteristics, in particular, the linear single fraction (ls), long chain (lc), short chain (sc), and others. The effects of different treatments on SSP chains from honeydew melon could be analysed at nanoscale via AFM images. The colour bar legends (in the web version) beside the image are scales for z-values, which represent the height of the scanned samples (Zhang et al., 2008). The morphologies of SSP chains were quite similar between control groups and treatment groups at day 1. All of the pectins had branching structures, long chains, and linear single fractions. However, there were slightly more short chains in control group

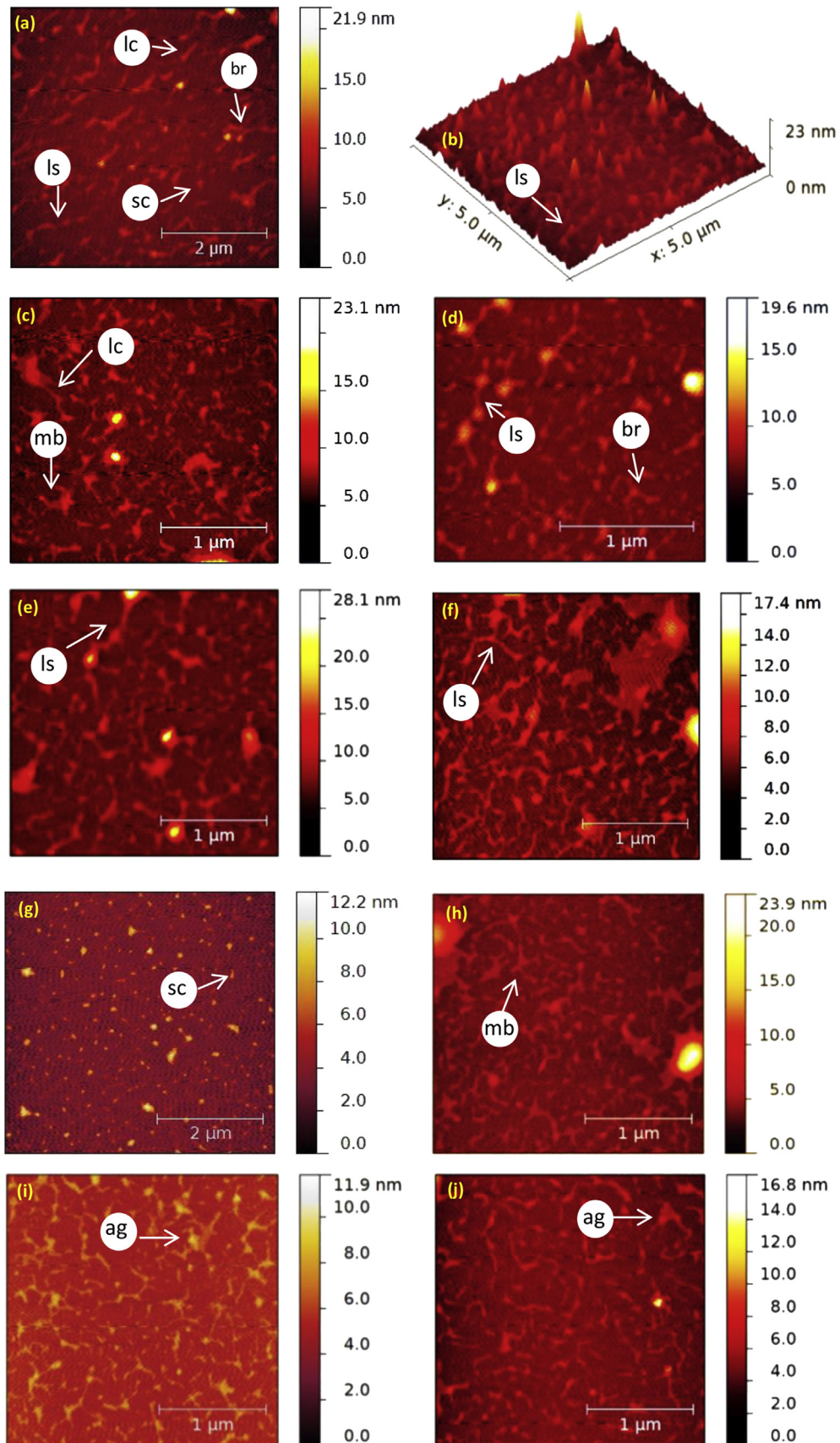


Fig. 5. AFM images of SSP chains from honeydew ^a. (a) before treatment; (b) its corresponding 3-dimensional image; (c) control group at day 1; (d) 2% chitosan group at day 1; (e) 1% CaCl₂ group at day 1; (f) combined treatment group at day 1; (g) control group at day 7; (h) 2% chitosan group at day 7; (i) 1% CaCl₂ group at day 7; (j) combined treatment group at day 7; (k) control group at day 13; (l) 2% chitosan group at day 13; (m) 1% CaCl₂ group at day 13; (n) combined treatment group at day 13. ^a Note: lc: long chain; sc: short chain; ls: linear single fraction; br: branching structure; mb: multiple branched chain; cp: cleavage point; ag: aggregates.

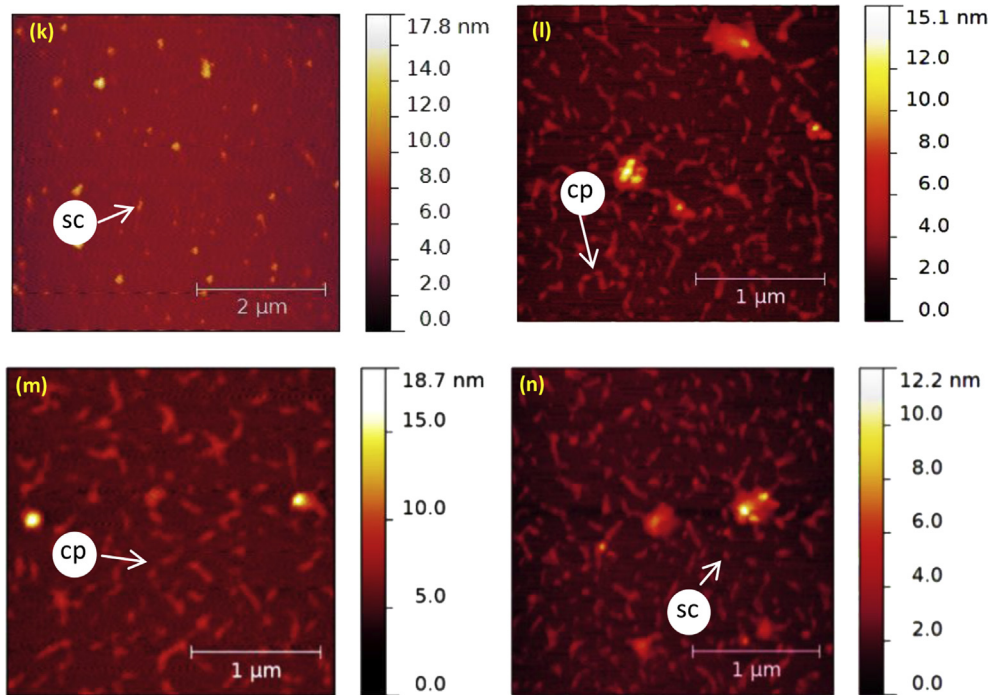


Fig. 5. (continued).

(Fig. 5c) at day 1 where most of the chains were randomly distributed instead of crosslinking with each other. On the contrast, in treated groups (Fig. 5d–f), a lot of chains linked with other chains. These linkages might contribute to the strengthening of cell wall structures (Yang et al., 2009). Besides, pectin morphologies between control group and treatment groups at day 7 (Fig. 5g–j) and day 13 (Fig. 5k–n) were significantly different. In particular, few or no obvious branched chains, long chains, aggregates were observed in control groups instead, most of the pectin chains were shortened and degraded significantly. As a comparison, obvious and significantly more branched structures, multiple branching structures, long chains, linear single fractions were present in all treatment groups, indicating that degradation of pectin molecules was associated with the texture breakdown of fruit tissue (Chen et al., 2011; Liu et al., 2009). In addition, short chains and detached branch structures increased, while long chains and linear single fractions decreased with storage for all groups, possibly due to the depolymerisation of pectin chains by enzymes especially pectinase (Chen et al., 2013).

3.5.2. Quantitative results

Apart from the qualitative information on SSP chains obtained for AFM analysis, quantitative results of AFM images can provide further detailed information to elucidate the modification and characteristics of pectin chains (Liu et al., 2009). As shown in Fig. 6, frequencies of length and width of SSP chains were determined, which represents the number of times the specific range of length and width presented (Zhang et al., 2008).

As shown in Fig. 6a, the lengths of SSP chains from honeydew before treatment were mostly within the range of 401–500 nm (33%), followed by 301–400 nm (22%) and 501–600 nm (28%). At day 7 (Fig. 5c), the control group contained more percentages of SSP short chains. The frequencies of length were as follows: 201–300 nm (33%), 101–200 nm (27%) and shorter than 100 nm (6%), which were significantly higher than all of the treatment groups, indicating the preventive effect of coating against

shortening and degradation of SSP chains. Because treatment groups correspondingly had better firmness than control group, the SSP quantitative information suggested SSP chains are closely related to honeydew's firmness. Degradation of SSP chains in all groups became significant at day 13, where the frequency of long chain lengths, especially those longer than 400 nm long were very limited (Fig. 6d). And none contained SSP chains longer than 600 nm. Coating treatment protected SSP chains from degradation because even though pectin degraded in treatment groups, the rate of degradation was much slower than that of control groups. Specifically, all of the treatment groups had significantly higher frequency of length in 301–400 nm at day 13 (Fig. 6d), while control group contained none of the pectin chains within this range. Instead, most of the pectin lengths in control group were within range of 101–200 nm, 201–300 nm and some even less than 100 nm. Combined these with texture results supported the correlation between tissue softening and shortening of pectin chain lengths (Chen et al., 2011, 2013; Yang et al., 2009; Zhang et al., 2008).

Apart from length, SSP chain width was also characterised by AFM. As shown in Fig. 6e, the widths of SSP chains from honeydew before treatment were mostly within the range of 51–60 nm (44%). The significant degradation of SSP at day 7 and day 13 (Fig. 6g and h), caused the widths of SSP chains from all groups to decrease. For instance, highest frequency of widths decreased to the range of 31–40 nm, followed by 21–30 nm at day 7. At day 13, higher frequencies of widths were found in categories 21–30 nm and short than 20 nm, respectively.

The shortening and depolymerisation, hydrolysis and solubilisation of pectin chains were largely attributed to increased activity of pectin-degrading enzymes (Chen et al., 2013). Based on current results, we propose that chitosan and calcium chloride coating treated groups extend the shelf-life of fresh-cut honeydew melon by maintaining the integrity of SSP molecules due to synergistic effect of both chitosan and calcium ions via strengthening molecular bonding between SSP molecules and chitosan protonated

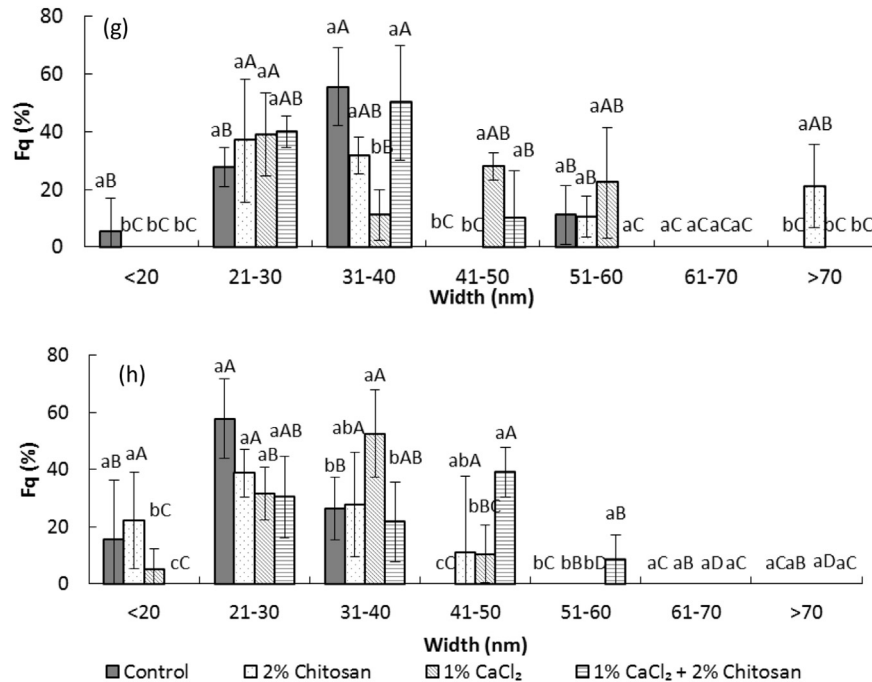


Fig. 6. (continued).

amino groups, and SSP and calcium ions from calcium salt (Chen et al., 2011; Goy et al., 2009). The AFM results provided direct evidence that the addition of calcium ions and chitosan was able to maintain and improve firmness by crosslinking pectin molecules together, strengthening the interaction and chemical bonding between pectin molecules, and thus preventing fruit texture degradation (Qi et al., 2011). Therefore, we hypothesised that the physicochemical properties, especially firmness information of fruits are correlated with cell wall polysaccharides tested by AFM. This correlation provides an even better approach on studying the effect of edible coating on fruit characteristics.

4. Conclusions

Treatments with 2% chitosan, 1% CaCl₂ and combination of 2% chitosan and 1% CaCl₂ significantly reduced weight loss, improved firmness, delayed colour changes, and inhibited microbial growth on fresh-cut honeydew melon during storage as compared to control group. The best delay in quality deterioration was found in the combined chitosan and calcium chloride treatment, with the shelf-life of fresh-cut honeydew successfully extended. The role of chitosan coating and calcium treatments in delaying the degradation of pectin molecules and maintaining the integrity of cell wall structure was elucidated in detail via nanostructural analysis of SSP chains by AFM, from morphological qualitative analysis to quantitative analysis on SSP length and width.

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