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ORIGINAL PAPER

Effect of Vacuum Impregnation Combined with Calcium Lactate on the Firmness and Polysaccharide Morphology of Kyoho Grapes (*Vitis vinifera* x *V. labrusca*)

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Abstract The effects of vacuum impregnation (VI) with 2% calcium lactate treatment on the VI properties (obtained from hydrodynamic mechanism and deformation–relaxation phenomena models), firmness, and pectin of Kyoho grapes were investigated. Fruit pectin was analysed by atomic force microscopy (AFM). VI was applied for 10–35 min at 25–45 °C and 5 kPa. The maximum values of effective porosity, ε_e (0.606%), and volume fraction, X (0.588%), occurred at 35 °C when the VI time was 15 min. No change was observed in the volumetric deformation ($\gamma \approx 0$) of the grapes after the impregnation. The firmness significantly increased at 35 °C VI (from 12.93 to 14.47 N). According to the AFM results, calcium mainly inhibited the degradation of chelate-soluble pectin and sodium carbonate-soluble pectin short branches during the VI. Under the studied conditions, the validity of VI to incorporate calcium into fruit to improve the quality of grapes was verified, and a final corresponding product was obtained by VI.

Keywords Vacuum impregnation · Grape · Firmness · Calcium · Pectin · Nanostructure · Atomic force microscopy (AFM)

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Introduction

Kyoho grapes (*Vitis vinifera* × *V. labrusca*), a typical nonclimacteric fruit with excellent organoleptic properties, are popular fruit cultivars in China. Nevertheless, their economic value is mainly limited by the characteristic of being perishable. Traditional storing methods such as refrigeration are insufficient to maintain the quality and prolong the storability (Valero et al. 2013). Coating effect is limited because it is difficult to diffuse the coating materials into the interior of the fruit (Chen et al. 2011; Chong et al. 2015). Therefore, it is critical to control the softening and browning to extend the shelf life of postharvest grapes.

Vacuum impregnation (VI) has been verified as a useful tool by rapidly and directly introducing different compounds into the matrix of food tissue to improve food's quality and extend its shelf life. The compounds are cryoprotectants, inhibitors of browning, enzymes, and probiotics (Krasaekoopt and Suthanwong 2008; Martínez-Monzó et al. 1998; Sapers et al. 1990; Yusof et al. 2016). These compounds have various advantages such as improving the nutrition values, extending shelf life, and modifying sensory attributes. Because of VI, the texture of the raw material is protected or even improved (Radziejewska-Kubzdela et al. 2014). Moreover, studies on the applications of VI to some fruits and vegetables validated the feasibility and validity of the coupling of hydrodynamic

mechanism and deformation–relaxation phenomena (HDM-DPR) model (Fito et al. 1996; Gras et al. 2002; Salvatori et al. 1998). The model to estimate the impregnation properties such as the volume changes at the end of VI (γ) and effective porosity (ε_e) affects the volume fraction of the initial sample impregnated by the external liquid (X) (Gras et al. 2003). Nevertheless, the effect of temperature and vacuum time on the impregnation parameters and texture of fruits is not well-known. Furthermore, the in-depth relationship between the impregnation and textural properties in fruits has not been fully elucidated.

Calcium is an essential mineral present in plant cell walls; it crosslinks the pectin polysaccharide chains and plays an important role in maintaining the fruit texture (Chen et al. 2011; Lai et al. 2013). It also maintains the membrane integrity and increases the cell rigidity (Aghdam et al. 2012). A frequently described method to improve texture and limit softening is the pretreatment of raw materials by introducing calcium ions into the tissue (Radziejewska-Kubzdela et al. 2014). In this regard, different types of calcium sources (calcium citrate, calcium chorine, calcium lactate, etc.) have been used for decay prevention and quality maintenance of fresh fruits (Martín-Diana et al. 2007, Kou et al. 2014). During a low temperature storage, the combined application of calcium chloride and gum arabic has shown to be an effective way to preserve the quality of mango fruit (Khaliq et al. 2015). Because of the activation of phenylpropanoid–flavonoid pathways in the cherry flesh, calcium chloride enhanced the antioxidant capacity of cherry (Aghdam et al. 2013). In addition, the calcium lactate treatment reduced the respiration rate and improved the firmness of persimmon slices (Almela et al. 2015). A comparison of calcium lactate and calcium chloride indicated that calcium lactate can be used for the shelf life extension of fresh-cut cantaloupe, and it led to a higher firmness without undesirable bitterness (Luna-Guzmán and Barrett 2000). Moreover, calcium lactate had shown antimicrobial effects (Aguayo et al. 2008; Pereira et al. 2010). Therefore, the substitution of calcium chloride with calcium lactate has a great potential.

The aim of this study was to evaluate the effect of VI with calcium lactate on the impregnation properties, mechanical properties, and polysaccharide morphology of the grapes. The feasibility of VI with calcium lactate to maintain the quality of grapes was determined by comparing the predicted and measured values of calcium content in the fruits. Moreover, the nanostructures of water-soluble pectin (WSP), chelate-soluble pectin (CSP), and sodium carbonate-soluble pectin (SSP) in the grapes were visualised and characterised by atomic force microscopy (AFM) in order to elucidate the detailed effects of calcium lactate on pectin after VI. The results provide the fundamental changes in the impregnation properties of fruit pectin with the textural changes in postharvest grapes during the VI.

Materials and Methods

Fruit Materials and Impregnation Solution Preparation

Grape berries of the Kyoho variety (16.19 ± 0.89°Brix) were freshly harvested from a vineyard of Zhengzhou, Henan, China. The grapes were transported at 4 °C to our laboratory within 4 h after the harvest. The clusters were selected based on a similar degree of ripeness by the apparent fruit quality (colour, size, and firmness). Approximately 2800 fruits (~18 kg) were selected for the experiment.

To minimise mass diffusive phenomenon other than HDM during the VI, an isotonic solution (IS) that contains sucrose and calcium lactate was used in the experiment. The solution concentration was quantified in °Brix using a refractometer. The density (ρ_{IS}) of the impregnation solution was measured using a pycnometer. The mass ratio of sample to solution was kept at 1:3. All the measurements were carried out in triplicate independently.

VI with Calcium Lactate

The same calcium levels (0 and 2% (w/w) of calcium lactate) were dissolved in an IS (≈16 °Brix), and VI was carried out in a jacketed chamber at a constant temperature (25, 30, 35, 40, and 45 °C). VI proceeded in the following steps: (0) sample submersion in the solution at time t_0; (1) a vacuum pressure of 5 kPa (p_1) was applied to the system for a short time t_1 = 10, 15, 20, 25, 30, and 35 min; (2) the atmospheric pressure (p_2) was gradually restored within 10 s, and the sample was maintained for 10 min (Betoret et al. 2015). The vacuum pressure was fitted to obtain a constant compression ratio ($r \approx p_2/p_1$) equal to 20 in the experiments. The capillary term (p_c/p_1) can be neglected for vacuum pressure lower than 40–60 kPa (Fito et al. 1996).

After the impregnation treatments, the adhering solution was removed from the surface with filter paper; then, the fruit was weighed. The initial sample weight (M_0) and final sample weight (M_t) were measured to determine the impregnated sample volume fraction (X) using Eq. (1). The volume of the samples was measured at the beginning ($V_0 = M_0/\rho_0$) and end of VI ($V_t = M_t/\rho_t$) to determine the deformation (γ) levels in terms of the volume fractions of sample using Eq. (2) (ρ_0 and ρ_t: apparent densities at initial and time t, respectively). The effective porosity (ε_e) of the sample available to the HDM action was calculated using the coupled HDM-DPR model. (Eq. (3)) (Salvatori et al. 1998).

$$X = \frac{M_t - M_0}{\rho_{IS} V_0} \quad (1)$$

$$\gamma = \frac{V_t - V_0}{V_0} \quad (2)$$

$$X-\gamma = \frac{\varepsilon_e(r-1)-\gamma}{r} \quad (3)$$

where, M_0 and M_t represent the mass of the grapes before and after VI treatment; ρ_{IS} is the density of the impregnating solution; V_0 and V_t are the volume of grapes before and after impregnating, respectively; and r is the compression ratio in the VI process.

Physicochemical Properties of Fruits

Soluble solid content (SSC) and titratable acidity (TA) were determined in juice obtained from 20 grapes per treatment. A refractometer (WYT-J, Sichuan, China) was used to measure the SSC (°Brix). TA was titrated with 0.1 M NaOH using a titrometer. The moisture content of the sample was quantified by drying in a vacuum oven at 60 °C until a constant weight was obtained (Salvatori et al. 1998). The ripeness index was calculated as the ratio of the SSC to TA. The apparent density (ρ_a) was determined using the pycnometer method with an IS as described by Salvatori and others (Salvatori et al. 1998). The actual density (ρ_r) was estimated from the water mass fraction, X_w (Eq. (4)). The porosity ε of the samples was calculated using these values according to Eq. (5).

$$\rho_r = 1590 \times (1 + 0.590 \times X_w)^{-1} \quad (4)$$

$$\varepsilon = \frac{\rho_r - \rho_a}{\rho_a} \quad (5)$$

where, ρ_r and ρ_a represent the actual density and apparent density of grapes, respectively; X_w is the water mass fraction; and ε is the sample porosity.

Firmness

The firmness of grapes was measured using a TA-XT2i texture analyser (Stable Micro Systems Ltd., Godalming, Surrey, UK). The operating parameters were set as follows: load cell = 40 kg, pretest speed = 3.0 mm/s, test speed = 1.0 mm/s, post-test speed = 3.0 mm/s, compression degree = 30%, time = 3.0 s, and trigger force = 5.0 g (Wu et al. 2008). For each condition group, 20 grapes were measured individually to arrive at a representative result.

Determination of Calcium Content

The mass fraction of the impregnated calcium (x_c) in the sample after the VI application may be estimated using Eq. (6) (Fito, Chiralt, Betoret et al. 2001a). An assumption was made to obtain the predicted values. The limiting factors for VI are the calcium concentration in the impregnation solution (C) and the effective porosity of the sample (Fito, Chiralt, Barat et al. 2001b). After the impregnation, the samples were digested by wet digestion, and calcium was quantified using an ICP-AES (iCAP 6000, Thermo Fisher Scientific, Shanghai, China). The reliability of the model was validated by comparing the predicted and measured values.

$$x_c = \frac{X \cdot C}{X\rho_{IS} + \rho_0} \quad (6)$$

where C is the calcium concentration in the impregnation solution; x_c is the mass fraction of the impregnated calcium in the sample after the VI.

Pectin Extraction

Three types of pectin (WSP, CSP, and SSP) were extracted using the literature method (Chong et al. 2015; Mierczyńska et al. 2015; Yang, Lai, et al. 2006a, Yang, Feng, et al., 2006b). Approximately 10 g flesh was boiled in 200 mL ethanol (80%, *v*/*v*) for 20 min. The mixture was filtered after cooling to room temperature, and the same procedure was repeated twice. Then, the solid residue was incubated in 50 mL DMSO/H_2O (9:1 *v*/*v*) at 4 °C overnight. After the filtration, the residue was dipped in a 200-mL chloroform/ethanol solution (2:1 *v*/*v*) for 10 min and then washed with 200 mL acetone until the total whitening. The residue was collected as the cell wall material (CWM).

WSP was extracted from the CWM by suspending it in 10 mL ultrapurified water for 4 h of shaking at 25 °C, followed by centrifugation at 10,000*g*, 4 °C for 10 min. (Shanghai Anting Scientific Instrument Factory, Shanghai, China). The above procedure was further repeated twice before the residue was resuspended in 10 mL of 50 mM CDTA (Sinopharm Chemical Reagent, China) for CSP extraction. After the extraction was repeated twice, the supernatants were pooled as the CSP. Subsequently, the residue was resuspended in 10 mL of 50 mM Na_2CO_3 containing 2 mM CDTA, shaken, and centrifuged three times as above. All the three supernatants were collected as SSP.

AFM Analysis

The nanostructures of WSP, CSP, and SSP were analysed using the methods used previously (Kirby et al. 2008; Lai et al. 2013; Yang 2014; Zhang et al. 2016). About 10 μL of the diluted pectin solution at a suitable concentration (10 μg/mL) was pipetted onto a freshly cleaved mica sheet surface. The mica surface was dried using an ear syringe ball. In the AFM, the cantilever used was a Si_3N_4 scanner with the ability to scan a 12 μm × 12 μm area, with a resolution of 0.1 nm in vertical, and the scan rate was ~0.5–2 Hz.

The images were analysed offline using an AFM software. The width and height of the sample were determined by section analysis. The width (*W*) and height (*V*) were calculated by

horizontal and vertical distances of chains using the software, respectively (Chen et al. 2013; Feng et al. 2016a, b; Feng et al. 2017; Yang, Lai et al. 2006a, Yang, Feng et al. 2006b; Yang 2014). The number of times a particular chain width was observed was recorded as the frequency (Fq) (Liu et al. 2009). At least 10 images were recorded from each sample to obtain credible statistical results.

Statistical Analysis

The results were expressed as means ± standard deviation. Each treatment was performed in triplicate. The statistical analysis of data was carried out using the statistical software SPSS to determine the significant differences between the groups. Comparisons that yielded $P < 0.05$ were considered significant.

Results and Discussion

Physicochemical Properties of Fresh Grapes

The physicochemical properties of fresh grapes used in this study are shown in Table 1. The results were the average of those obtained for 20 samples. In all the cases, the ripeness index corresponds to the fruits with a firm texture and favourable organoleptic properties. The ripeness index of grape was 160.89 unit. The fruit porosity (ε) was measured from the lacuna in the fruit tissue and reflected the maximum interspace impregnated with an IS (Krasaekoopt and Suthanwong 2008). The porosity of Kyoho grapes (2.17%) was close to the result of Kiwi fruit (*Hayward*) (2.3%) (Salvatori et al. 1998). In addition, the variation of X was associated to the gas expansion and the external solution that penetrated the food. However, the volumetric fraction of impregnated solution was lower than the actual amount of external solution that penetrated the food (Laurindo et al. 2007).

Table 1 Physicochemical properties of fresh 'Kyoho' grapes

Property	Result
Moisture content (%)	81.22 ± 1.03
Soluble solid content (°Brix)	16.25 ± 0.87
Acidity (%)	0.10 ± 0.00
Ripeness index (°Brix/%)	160.89 ± 7.01
Apparent density (kg/m^3)	1055.39 ± 9.77
Real density (kg/m^3)	1074.91 ± 4.42
Fruit porosity (ε) (%)	2.17 ± 0.95

VI Properties of Grapes

The VI properties such as volume fraction (X) (Fig. 1a) and effective porosity (ε_e) are shown in Table 2; they indicate the impregnation level of the sample. An ANOVA considering the factor treatment showed that the vacuum time (t_1) did not significantly affect ($P > 0.05$) the volumetric deformation (γ), consistent with that previously reported for some fruits (Salvatori et al. 1998). Moreover, almost no change occurred at γ (≈0) (the volume of the sample remained almost the same as the initial sample) of the grapes after the impregnation. This result can be explained by their wide intercellular pores and rigid cellular tissue, which hardly hindered the fluid flow (Krasaekoopt and Suthanwong 2008).

Nevertheless, a significant HDM action was observed in the sample because a significant increase of X (from 0.398 to

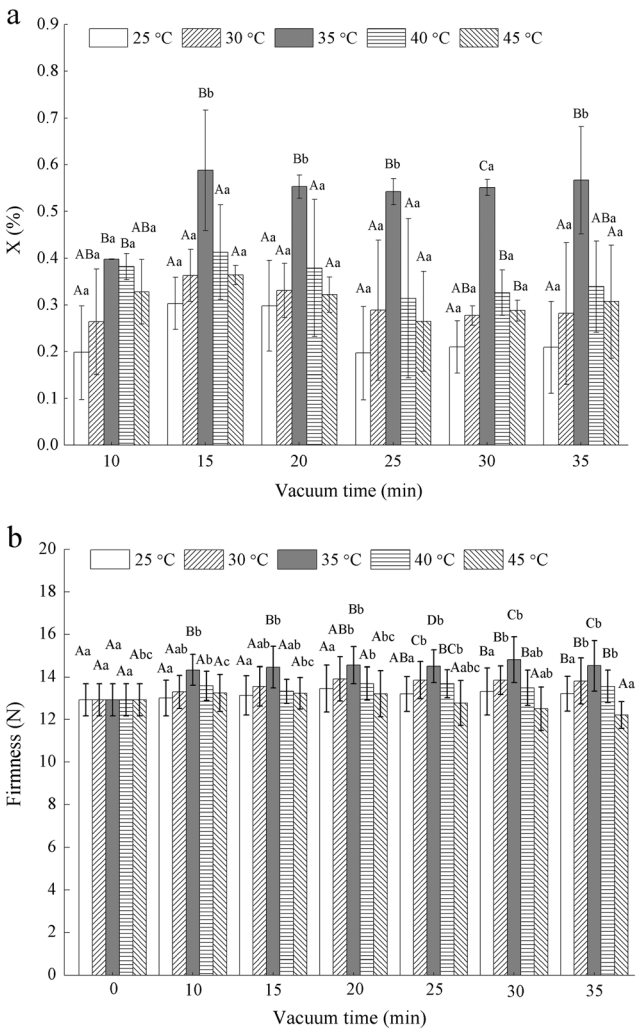

Fig. 1 Effect of temperature and vacuum time on firmness and volume fraction (X) of grapes. Values are presented as mean ± SE ($n = 20$). Within the same vacuum time, different capital letters indicate significant differences, while for the same temperature but different vacuum time, different lowercase letters indicate significant differences ($P < 0.05$)

Table 2 Effect of time and temperature of vacuum impregnation (VI) treatment on the effective porosity (ε_e) in the grapes

t_1 (min)	ε_e (×10 m^3 of internal gas/m^3 initial sample)				
	25 °C	30 °C	35 °C	40 °C	45 °C
10	$2.09 \pm 0.78^{a,A}$	$2.88 \pm 0.27^{ab,A}$	$3.96 \pm 0.54^{b,A}$	$4.00 \pm 0.36^{b,A}$	$3.44 \pm 0.29^{ab,A}$
15	$3.29 \pm 0.57^{a,A}$	$3.81 \pm 0.26^{a,A}$	$6.06 \pm 0.12^{b,B}$	$4.34 \pm 0.41^{a,A}$	$3.82 \pm 0.30^{a,A}$
20	$3.03 \pm 0.11^{a,A}$	$3.36 \pm 0.26^{a,A}$	$4.40 \pm 0.10^{b,B}$	$3.97 \pm 0.32^{a,A}$	$3.39 \pm 0.26^{a,A}$
25	$1.86 \pm 0.48^{a,A}$	$2.84 \pm 0.36^{a,A}$	$5.68 \pm 0.36^{b,B}$	$3.27 \pm 0.20^{a,A}$	$2.77 \pm 0.15^{a,A}$
30	$2.01 \pm 0.26^{a,A}$	$2.80 \pm 0.30^{ab,A}$	$4.95 \pm 0.48^{c,B}$	$3.44 \pm 0.21^{b,A}$	$3.00 \pm 0.61^{b,A}$
35	$2.09 \pm 0.31^{a,A}$	$2.83 \pm 0.12^{a,A}$	$5.95 \pm 0.62^{b,B}$	$3.55 \pm 0.31^{ab,A}$	$3.22 \pm 0.15^{a,A}$

Different superscript lowercase letters in the same row and different superscript capital letters in the same column indicate significant differences at $P < 0.05$

0.588) was obtained at 35 °C when the vacuum time was increased from 10 to 15 min; value of X then reached a plateau (Fig. 1a). This result indicates that the filling of the pores was affected by temperature and can be attributed to a fast mass transfer phenomenon.

Effective porosity (ε_e) is defined as the total initial volume in the sample tissue available to the external liquid (Fito et al. 1996). Simultaneously, it reflects the sample behavior during VI. Under these operating conditions, the value of ε_e was closely parallel to the X value (Table 2), consistent with that reported for guava and papaya (Krasaekoopt and Suthanwong 2008). The effective porosity of grape significantly increased with time, indicating that at least 15 min VI is essential to reach the mechanical equilibrium of sample. To compare the fruit porosity (ε) with the effective porosity (ε_e), the $\varepsilon_e/\varepsilon$ ratio was defined as an index of the internal volume of the pores available for HDM. The results indicate that ε_e was less than ε in all the cases. This might have been caused by the structural modifications or capillary effect; the free volume was not entirely filled. The $\varepsilon_e/\varepsilon$ ratio was within the range 0.090–0.279.

The above mentioned results indicate that Eq. (3) can be modified as Eq. (7):

$$X = \varepsilon_e \frac{r-1}{r} \tag{7}$$

Effect of VI with Calcium on Fruit Firmness

The structure and mechanical properties of the grapes were probably affected by the external liquid with a certain composition and properties, which penetrated into the extracellular space of the tissue (Chafer et al. 2003; Guillemin et al. 2008). The fruit texture is closely related to the tissue calcium levels. The IS with calcium infiltration resulted in a firming effect under suitable conditions (Fig. 1b). The firmness of the grapes almost remained stable when the vacuum time was >15 min. The results showed that the firmness of the samples significantly increased at 35 °C. However, the firmness of the samples decreased when the fruits were impregnated >15 min at 45 °C. This is because heat leads to an irreversible damage of cellular tissue. Besides, the 35 °C calcium-treated group was significantly firmer than the other groups at the same vacuum time. This is probably because the sample reached a higher impregnation level (Table 2 and Fig. 1a) under this operating condition. Interestingly, the firmness increased to a level even greater than that at the harvest after the VI. This phenomenon can be attributed to the increase in calcium content and moisture loss in fruit cells, increasing the force needed to break the fruit peel (Chen et al. 2011). Therefore, the calcium content in the samples and nanostructural information of pectin at 35 °C for VI treatment groups were investigated.

Comparison of the Predicted and Measured Values of Calcium Content for VI Grapes

During the impregnation, the calcium ion transport inside the fruit was caused by the vacuum pulse. Figure 2 shows a

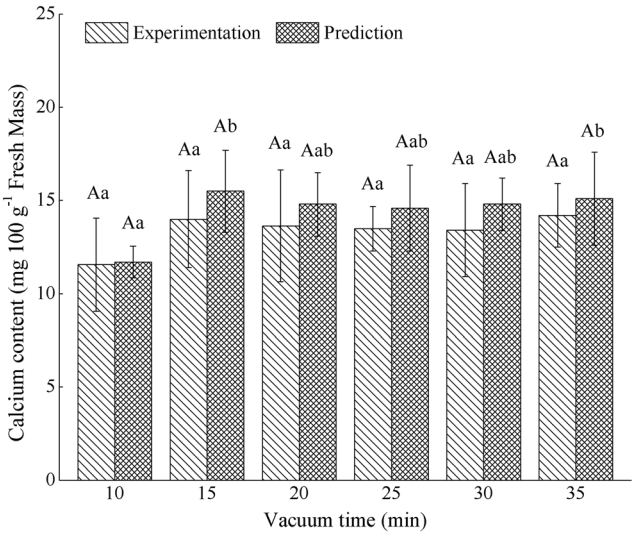

Fig. 2 Comparison of calcium content between the experimental and predicted results for grapes. Within the same vacuum time, different capital letters indicate significant differences, while for the same temperature but different vacuum time, different lowercase letters indicate significant differences ($P < 0.05$)

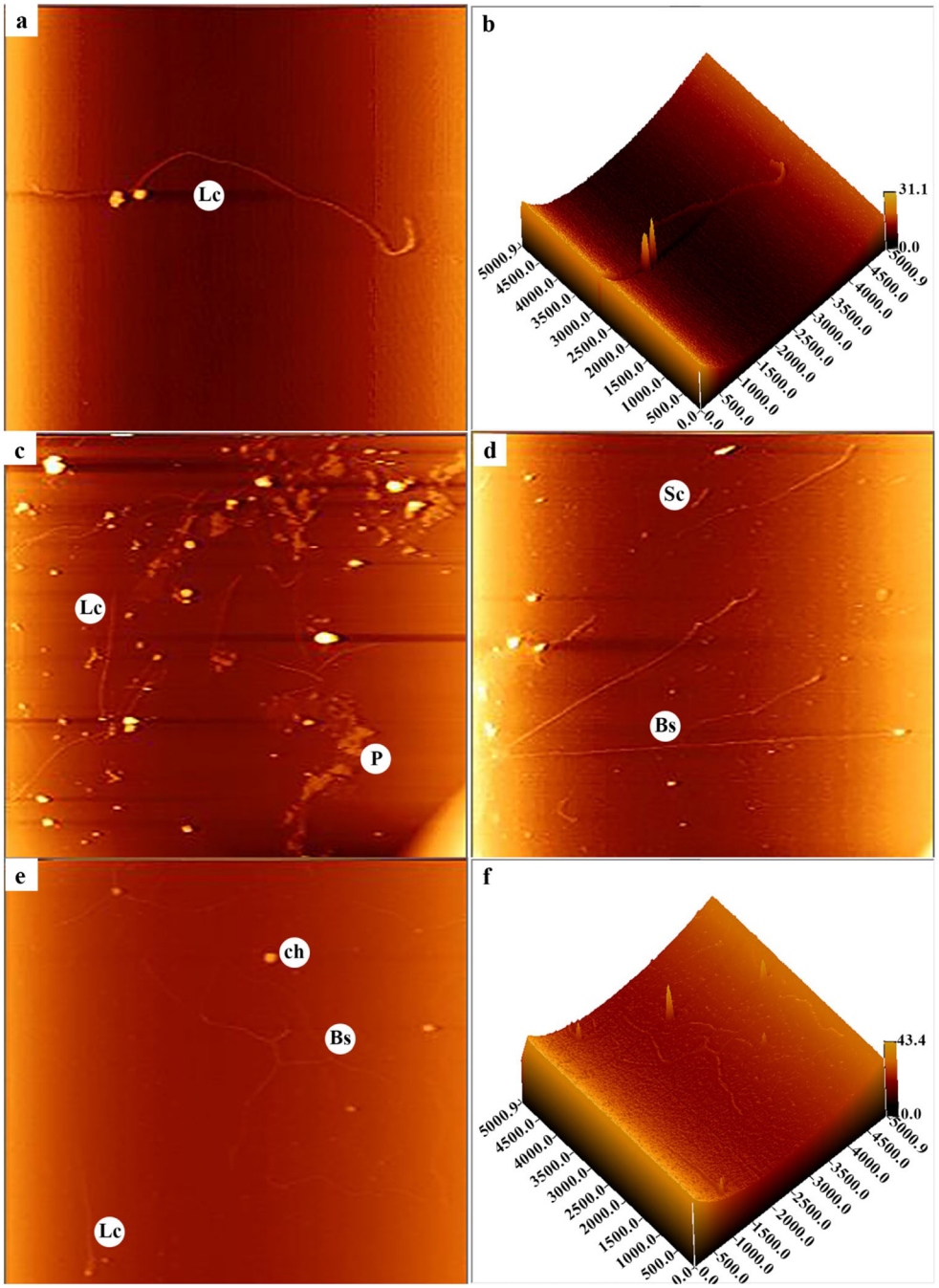

Fig. 3 Atomic force microscopy (AFM) images of pectins from 'Kyoho' grapes after vacuum impregnation (VI) with calcium lactate treatment. **a**, **b** Water-soluble pectin (WSP) images from grapes of CK group. **c** WSP images from grapes of VI with 2% calcium lactate for 10 min (t_1) at 35 °C. **d** WSP images from grapes of VI with 2% calcium lactate for 35 min (t_1) at 35 °C. **e**, **f** Chelate-soluble pectin (CSP) images from grapes of CK group. **g** CSP images from grapes of VI with 2% calcium lactate for 10 min (t_1) at 35 °C. **h** CSP images from grapes of VI with 2% calcium lactate for 35 min (t_1) at 35 °C. **i**–**j** Sodium carbonate-soluble pectin (SSP) images from grapes of CK group. **k** SSP images from grapes of VI with 2% calcium lactate for 10 min (t_1) at 35 °C. **l** SSP images from grapes of VI with 2% calcium lactate for 35 min (t_1) at 35 °C. Image size: 5.00 × 5.00 μm^2. Note: *Lc* long chains; *Ls* linear single fractions; *P* polymers; *Sc* short chains; *Bs* branch structures; *ch* chelator, CDTA; *rp* releasing point of pectin released from the CDTA; *cp* cleavage points

comparison of the predicted and measured values of calcium content reached in grapes in the VI operation at 35 °C for 10–35 min. Values calculated using Eq. (6) were consistent with the experimental results when the vacuum time was increased. On the other hand, the calculation results obtained from the experiment paralleled the consequences of the X values. Clearly, the predicted values are similar to the experimental results. Moreover, the relative errors between them are almost

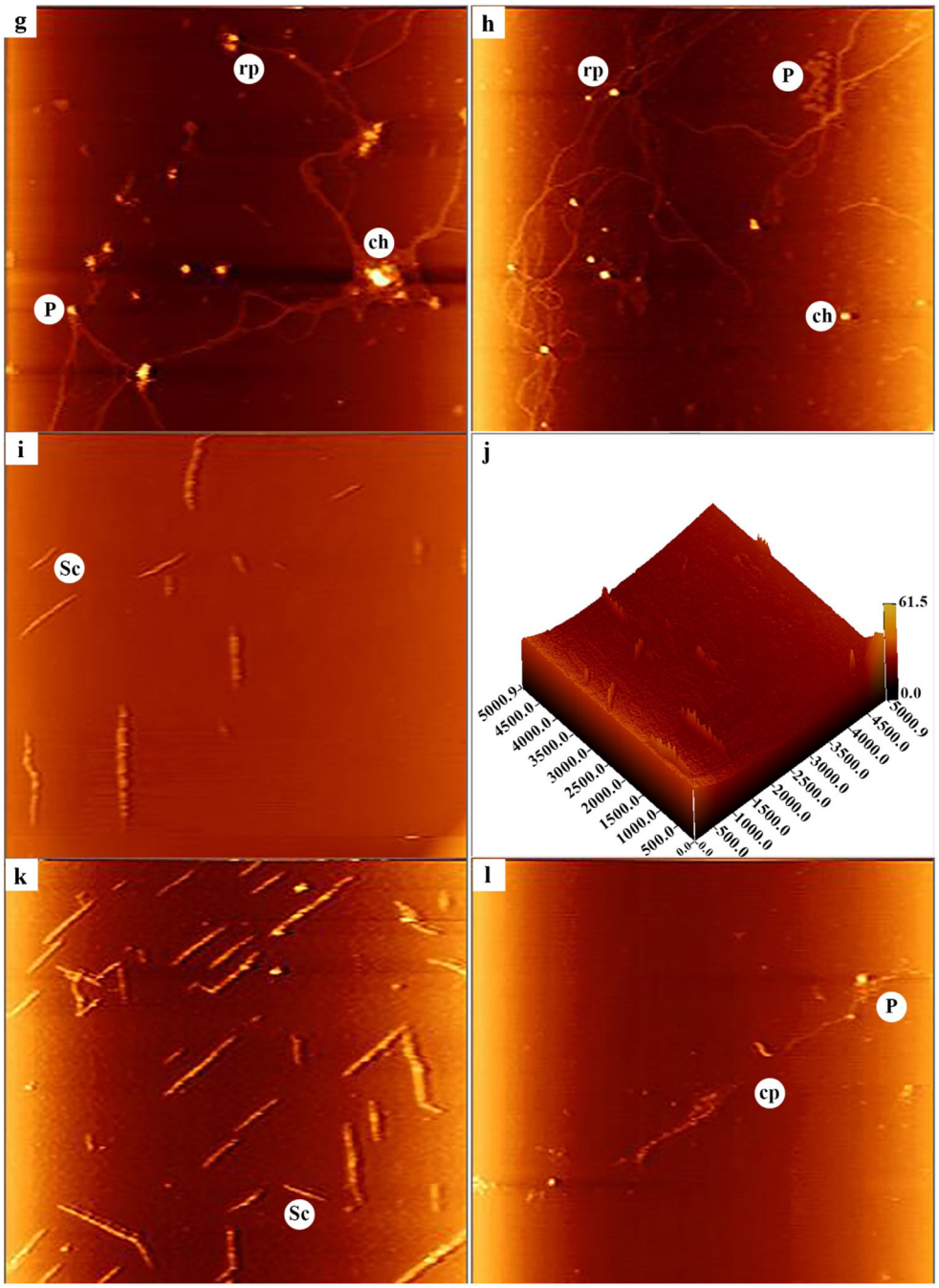

Fig. 3 (continued)

within 10%. Therefore, through the comparison between the predicted and experimental results, the feasibility and validity of VI to incorporate calcium into fruits to improve the quality of grapes were verified. To achieve the desired level in the final product, the required solution concentration of a determined component was calculated from the predicted value (Fito, Chiralt, Barat et al. 2001b). In addition, this process shows the promise of VI combined with salts and sanitising technologies for possible application in organic foods (Li et al. 2015; Yu et al. 2017; Yu and Yang 2017; Zhang and Yang 2017; Zhao et al. 2017) and in examining functional properties of fruits (Fu, Yang, Peh et al. 2015a, Fu, Yang, Lai et al. 2015b).

AFM Analysis

The changes in cell wall polysaccharides contribute to firmness changes, particularly the alteration of pectin nanostructures (Chen et al. 2011). Figure 3 shows the AFM images of the WSP, CSP, and SSP chains of grapes in the CK group, VI

(t_1 = 10 min) and VI (t_1 = 35 min) groups. The chain widths of the pectin were characterised by AFM (Fig. 4), showing the width range with the time (Zhang et al. 2008). Multiple pectin nanostructures and chain widths reflect the heterogeneity and complexity of the pectin structure in the cell wall fractions. The results indicated that VI treatment affected the qualitative morphology of pectin.

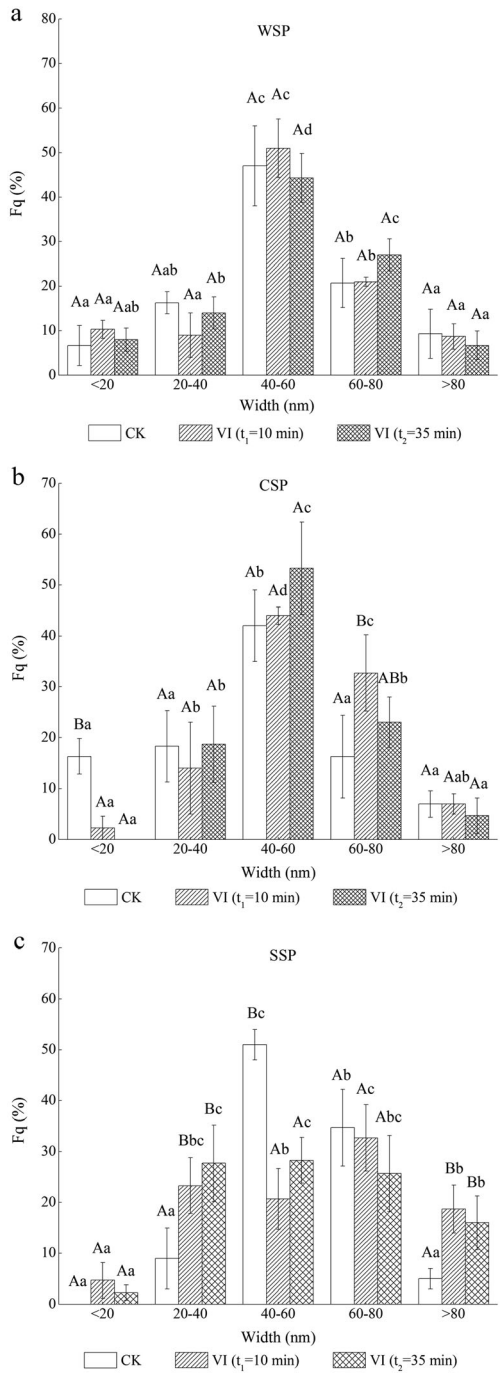

Fig. 4 Effect of vacuum impregnation (VI) with calcium lactate on quantitative width distribution of pectin chains of 'Kyoho' grapes. **a** Water-soluble pectin (WSP). **b** Chelate-soluble pectin (CSP). **c** Sodium carbonate-soluble pectin (SSP). For each pectin, within the same range of width, different capital letters indicate significant differences, while within the same treatment group, different lowercase letters indicate significant differences among the range of widths ($P < 0.05$)

Qualitative Results of Nanostructures

The quantitative information of pectin molecules was also shown by the AFM images. The WSPs of most of the grapes were linear chains (Fig. 3) with a small proportion of branches (Fig. 3c, d) or without branches (Fig. 3a), similar to the WSPs in strawberry and Chinese cherry (Chen et al. 2011; Lai et al. 2013). Furthermore, some polymers were also present. The 3D height images showed that the surfaces of WSPs were uneven (Fig. 3b).

The modifications of the width of CSP and SSP during the VI were more distinct than those of WSP. CSP is recognised as an ionically bound pectin and involved in complex structures. It maintains the structural integrity of tissue (Lara et al. 2004). As shown in Fig. 3, most of the CSP chains are multibranched, and large CSP polymers are always associated with the chelator, CDTA (ch). Rarely, single linear chains or short rods were observed. Even more aggregates existed in the VI treatment group than the control. Most of the SSPs were single linear chains or short rods, and only a few aggregates or large polymers were observed, as shown in Fig. 3. The results are in contrast to the previously reported SSPs of other fruits and vegetables (Liu et al. 2016; Yang 2014), probably because of the different properties of SSP in different fruits.

Quantitative Results of Nanostructures

The width of pectin chains changed accompanied by the degradation of cell wall polysaccharide. Compared to the CK group, the effect of calcium lactate on the width of WSP chains was not significant during the VI treatment (Fig. 4). The widths of the WSP chains were mainly within the range 40–60 nm. The frequency of width < 20 nm was almost equal to the width > 80 nm. Almost all the heights of the WSPs in the grapes were ~0.5–3.9 nm, which is similar to the jujubes (0.5–3 nm) (Yang et al. 2012).

As shown in Fig. 4, the widths of CSPs were significantly affected by VI with calcium treatment. The Fq values of the small-width chains (<20 nm) were 16.3% for the CK group; this was higher than that in the VI (t_1 = 10 min) treatment group (2.3%). However, the small-width chains even did not appear at VI (t_1 = 35 min) group. Moreover, the VI group had a higher Fq of larger width chains (60–80 nm) than the CK (16.3%). The results indicate that Ca^{2+} worked during the VI. Its mechanism involves the strengthening of the capability of cross-linking between

homogalacturonans, which were ionically cross-linked by calcium between the pectin molecules (Souty et al. 1995). Therefore, VI with calcium lactate inhibited the degradation or breakage of the side branches of CSP. For chain heights, mainly in the range 0.4–3.5 nm, no significant difference was observed among the different groups. The results are consistent with those reported previously for apricot fruits (Liu et al. 2009).

The widths of SSP from the CK group, measured by AFM, were mostly within the range of 40–60 nm (51%) (Fig. 4). The VI group had a significantly lower frequency of width within 20–40 nm than VI group. However, no significant difference was observed between both the VI (t_1 = 10 min) and VI (t_1 = 35 min) groups (23.3 and 27.6%, respectively). Moreover, the Fq of larger widths (>80 nm) from VI groups (18.7 and 16%) were higher than that of CK group (5%). This result indicates that the Fq of large width in the CK group decreased rapidly than those of the VI with calcium treatment groups. The trend was similar to the results of strawberries (Chen et al. 2011).

Conclusion

The impregnation time and temperature affect the VI properties (as obtained from the HDM-DPR model) such as the effective porosity (ε_e) and volume fraction of impregnated grapes (X). However, almost no change in the volumetric deformation (γ) of the grapes occurred after the VI. The high values of ε_e and X correspond to a greater firmness at 35 °C during the VI treatment. The calcium content determination indicated that it is possible to predict the calcium content in the samples from X and ε_e. Consequently, suitable conditions for the impregnation of grape were (i) vacuuming fresh grape with 2% calcium lactate for 15 min and (ii) a 10-min relaxation period at atmospheric pressure and 35 °C. These conditions are useful to achieve the maximum impregnation level and obtain a better quality using VI. Calcium mainly inhibited the degradation of CSP and SSP short branches during the VI from AFM results. The VI (t_1 = 10 min) group had a lower frequency (2.3%) of small-value chain widths for CSP than the CK group (16.3%) and a higher frequency (18.7%) of a large-value chain widths for SSP than the CK group (5%). Thus, the results suggest that VI with calcium lactate treatment is promising to improve the texture of grapes.

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