Ultrasonics - Sonochemistry 60 (2020) 104784

Contents lists available at ScienceDirect

Ultrasonics - Sonochemistry

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

Calcium permeation property and firmness change of cherry tomatoes under ultrasound combined with calcium lactate treatment

Lifen Zhang[a], Pei Wang[a], Xiaoyang Sun[a], Fusheng Chen[a,*], Shaojuan Lai[a,b], Hongshun Yang[c,d,*]

[a] College of Food Science and Technology, Henan University of Technology, Zhengzhou, Henan 450001, PR China
[b] College of Basic Medicine, Guizhou University of Traditional Chinese Medicine, Guiyang, Guizhou 550025, PR China
[c] Department of Food Science & Technology, National University of Singapore, Singapore 117543, Singapore
[d] National University of Singapore (Suzhou) Research Institute, 377 Lin Quan Street, Suzhou Industrial Park, Suzhou, Jiangsu 215123, PR China

ARTICLE INFO

Keywords:
Ultrasound
Calcium lactate
Pectin
Cherry tomato
Atomic force microscopy
Nanostructure

ABSTRACT

This study aimed to investigate the effect of ultrasound combined with calcium lactate (2%, w/v) treatment (U + Ca) on calcium permeation and firmness of cherry tomatoes. Calcium distribution and fruit pectin nanostructure were also analysed by transmission electron microscope (TEM) and atomic force microscopy (AFM), respectively. The firmness (31.45 N) was maintained when ultrasound energy density was 20 W/L for 15 min at 15 °C. The Ca content increased in U + Ca treated fruit. Meanwhile, the Peleg's model could be used to express the change of solid gain in cherry tomatoes under ultrasound treatment at 15, 20, and 25 °C. According to the AFM results, the width (≥ 40 nm) and length (≥ 2 μm) of chelate-soluble pectin (CSP) and sodium carbonate-soluble pectin (SSP) chains with large frequency was observed in U + Ca treated fruit. Under desirable conditions (15 °C, 15 min, 20 W/L), ultrasound combined with calcium lactate could maintain the quality of cherry tomatoes.

1. Introduction

Cherry tomatoes are one of the most popular fruit because of their rich nutrient content. The ascorbic acid content is higher than that in normal tomatoes. In addition, there are lycopene and glutathione in cherry tomatoes, which is benefit for cancer prevention [1]. However, being a climacteric fruit, postharvest rapid ripeness made it easily damaged and contaminated by microorganisms [2], thus resulting in huge economic losses [3]. Meantime, the total phenolic and ascorbic acid content decreased [4,5], which lead to the decrease of nutritive value. Thus, the need for treatment and storage technologies capable of controlling fruit quality is rising.

In the past decades, considerable work has been conducted to preserve the quality of cherry tomatoes. The most common preservation method is refrigeration. However, this is not sufficient to meet the market demand to extend the shelf life of fresh fruit. Calcium (Ca) is reported to play a key role in suppressing quality decline, preserving integrity, and reducing the permeability of the cell membrane in fruit [6]. Meanwhile, Ali, Abbasi, and Hafiz [7] reported that calcium can improve the quality of peach without compromising the food safety standards. Researchers reported that the physical properties of fruit treated with calcium chloride and calcium lactate can be effectively maintained, and the degradation of pectin and hemicellulose in the cell wall of fruit is also delayed significantly [8]. Calcium lactate treatment can reduce the respiration rate, improve the firmness, antimicrobial, and prolong the shelf life of fruit [9]. Furthermore, Ca^{2+} can inactivate the polygalacturonase (PG), which is responsible for the degradation of cell wall materials and component like pectin, and Ca^{2+} plays an important role in maintaining fruit quality. Therefore, the use of calcium lactate has a great potential [10].

Coating combined with advanced technologies, such as ultrasound, is one of the recent approaches for the prevention of fruit and vegetables. It has been reported that the progress of calcium penetration can be promoted by physical methods, leading to an increased Ca content in fruit tissue [11]. The Ca content in jujube fruit tissue was increased by treatment with ultrasound combined with calcium chloride, and the Ca distribution was also affected [12].

Ultrasound, as a mild, environmentally-friendly, and non-toxic technology, has been used to prolong the shelf life, to inactivate attached microbes, and to preserve the texture properties and sensorial quality of fruit [13,14]. The effect of ultrasound is based on its mechanical energy, thermal, and chemical effects [15]. The intense

* Corresponding authors at: Department of Food Science & Technology, National University of Singapore, Singapore 117543, Singapore (H. Yang). College of Food Science and Technology, Henan University of Technology, Zhengzhou 450001, Henan, China (F. Chen).
E-mail addresses: fushengc@haut.edu.cn (F. Chen), chmynghs@nus.edu.sg (H. Yang).

https://doi.org/10.1016/j.ultsonch.2019.104784
Received 21 June 2019; Received in revised form 16 August 2019; Accepted 7 September 2019
Available online 09 September 2019

pressure generated during cavitation process of ultrasound can increase the penetration of compounds from the extracellular to the intracellular environment [16]. In addition, the cavitation bubble collapsing induces mechanical degradation of pectin [17]. Pectin is distributed in the primary cell wall and middle lamella of all plant tissues, and plays an important part in fruit firmness and softening. Pectin depolymerisation was accompanied by softening of fruit during storage [18]. Meanwhile, ultrasound treatment also can affect the firmness of fruit. Studies on tomatoes treated with ultrasound showed that the firmness of the fruit can be affected by the parameters of ultrasound [14].

The effect of ultrasound combined with other methods in keeping the freshness of fruit and vegetables had been studied. It had been proved that ultrasound combined controlled relative humidity treatment can maintain the quality of straw mushroom and pineapple [19]. In addition to this, ultrasound combined with calcium chloride had a positive effect on the quality of jujube and strawberry during storage [12,20]. There is a little research on the calcium permeation under ultrasound, and also for the mechanism of ultrasound combined with calcium solution affecting the firmness of fruit.

To elucidate the permeation property of Ca under ultrasound combined with calcium lactate treatment, this research was aimed to study the distribution of calcium in pulp tissue cell and the solid gain model. The firmness of cherry tomatoes and nanostructure of pectin were also investigated to reveal the mechanism of ultrasound combined with calcium lactate treatment on fruit firmness. The results would provide a theoretical foundation for the application of ultrasound to preserve postharvest fruit.

2. Materials and methods

2.1. Materials

Cherry tomatoes (*Solanum lycopersicum* L. cv YuyiLiangjingjing) selected with a similar size (18 ± 1 g), colour and ripening stage (7–8, light red), were harvested in a commercial greenhouse in Zhengzhou, Henan, China. The physicochemical properties of cherry tomatoes were as follows: soluble solids content was 5.75 ± 0.50 °Brix, titratable acid was 0.24% ± 0.07, and water content was 93.78% ± 0.50. The fruit was transported to the lab in 2 h. Mechanically damaged or microbial infected fruit were discarded. The remainder (about 2160 cherry tomatoes) were randomly selected and divided into 72 groups (thirty fruit for each group), and stored at 5 °C for further treatment. Food grade calcium lactate (content 98%-101.0%) was purchased from Henan Jindan Lactic Acid Technology Co., Ltd. (Zhoukou, Henan, China).

2.2. Ultrasound combined with calcium lactate treatment

For the ultrasound combined with calcium lactate treatment (U + Ca group): cherry tomatoes were directly immersed in a sonicator bath equipped with a low-temperature thermostat (energy density 20 W/L, frequency 40 kHz, Xinzhi, SBL-10DT, Ningbo, Zhejiang, China) with 10 L calcium lactate solution (200 g dispersed in 10 L distilled water (2%, w/v) at temperatures of 15, 20, 25, 30, and 35 °C for 5, 10, 15, 20, 25, 30, 35, and 40 min, respectively. In addition, the effect of ultrasound energy density (16, 20, 24, 28, 32, and 36 W/L) on the fruit at 20 °C for 20 min was also investigated.

For calcium lactate treatment (Ca group), fruit were immersed in calcium lactate solution (2%, w/v) at temperatures of 15, 20, 25, 30, and 35 °C for 5, 10, 15, 20, 25, 30, 35, and 40 min, respectively. Fruit without any treatment were defined as fresh fruit.

2.3. Firmness

Firmness of the cherry tomatoes was determined using a Texture Analyser (Stable Micro Systems Ltd., Godalming, Surrey, UK) fitted with a 35 mm diameter probe according to Pinheiro, Alegria, Abreu, Gonçalves, Silva [14] and Xin, Chen, Lai, Yang [21] with some modifications. The operating parameters were set as follows: pre-test speed = 3 mm/s, test speed = 1 mm/s, post-test speed = 5 mm/s, compression degree = 20%, trigger force = 10 g. Twenty replicates randomly selected from each group were measured individually.

2.4. Microstructure of cherry tomato observed with scanning electron microscopy

A piece of cherry tomato flesh (3 mm length × 2 mm width × 1 mm thickness) at equatorial zone was excised by a blade. The pieces of flesh were fixed in 2.5% glutaraldehyde at 4 °C for 24 h, rinsed three times with phosphate buffer and each time for 15 min. Subsequently, the pieces were dehydrated in a series of ethanol solution (30%, 50%, 70%, 90%, 100%) for 10 min, then 100% acetone. The specimens dried with a freezer dryer and observed by a scanning electron microscopy (SEM) (FEI, Quanta250FEG, US) [22].

2.5. Determining the Ca content

Ca content was determined using the literature method [23]. Flesh from six cherry tomatoes was dehydrated in a dryer until it reached a constant weight (105 °C, 36 h), and then ground to a fine powder. About 0.5 g of dried sample was ashed in a muffle furnace. The ashed samples were digested in a mixture of concentrated HNO_3 and $HClO_4$ at a ratio of 4:1, and heated to remove excess digestion solution. The remaining component was diluted to 25 mL. The sample was then analysed using a TAS-990 atomic absorption spectrometer (General Analysis Beijing General Instrument Co., Ltd., Beijing, China). The Ca content was expressed in grams per kilogram of dry matter.

2.6. Distribution of Ca in pulp tissue cell

Distribution of Ca in pulp tissue cell was assayed by a transmission electron microscope (TEM) (Japan Electronics Corporation, JEM-2100, Japan). Piece of 2 × 4 mm was cut from pulp tissue and fixed in 3%-4% glutaraldehyde solution prepared in phosphate buffer (0.1 mol/L, pH 7.2, containing 20 g/L potassium pyroantimonate) at 4 °C for 48 h. Then sample was immersed in 10 g/L OsO_4 at 4 °C for 3 h. After dehydrated in a graded series of ethanol solutions, the sample was embedded in resin. The sample was sliced (about 70 nm), stained (50 g/L uranyl acetate and 20 g/L lead citrate) and observed by TEM at 80 kV [12].

2.7. Solid gain

Cherry tomatoes were dried in an oven at 105 °C until the weight did not change. The weight of the dried substance was noted [24]. Solid gain (ΔM) was calculated using the following formula:

$$\Delta M = \frac{m_t x_{st} - m_0 x_{s0}}{m_0} \quad (1)$$

where m_0 is the weight of the fresh fruit; m_t is the weight of fruit treated at different conditions; χ_{s0} is the solid content of the fresh fruit; and χ_{st} is the solid content of fruit treated at different condition. To better evaluate the change of solid gain, Peleg's model was used to model the solid gain change [25].

$$\Delta M_t = \Delta M_0 + \frac{t}{k_1\, t + k_2} \quad (2)$$

where ΔM_0 is the initial solid gain; ΔM_t is the solid gain of cherry tomatoes treated for t min. The fitting degree of the model was judged using the correlation coefficient (R^2), root mean square error (RMSE), and the mean relative percentage deviation modulus (E) [26].

$$RMSE = \frac{1}{N}\left[\sum_{i=1}^{N} (V_e - V_P)^2\right]^{0.5} \quad (3)$$

Table 1
Effect of ultrasound combined with calcium lactate treatment on the firmness (N) of cherry tomatoes.

Time (min)	Ultrasound temperature (°C)				
	15	20	25	30	35
0	30.61 ± 4.29$^{cd.A}$	30.61 ± 4.29$^{c.A}$	30.61 ± 4.29$^{b.A}$	30.61 ± 4.29$^{d.A}$	30.61 ± 4.29$^{e.A}$
5	31.33 ± 3.51$^{d.B}$	30.19 ± 4.37$^{c.B}$	30.82 ± 3.79$^{b.B}$	30.67 ± 4.45$^{d.B}$	27.06 ± 3.90$^{d.A}$
10	31.01 ± 3.80$^{d.C}$	29.17 ± 3.06$^{bc.BC}$	30.72 ± 2.80$^{b.C}$	29.50 ± 2.86$^{cde.BC}$	25.80 ± 3.79 $^{cd.A}$
15	31.45 ± 3.80$^{d.C}$	28.48 ± 3.85$^{bc.B}$	28.76 ± 3.90$^{ab.B}$	28.24 ± 3.00$^{bcd.B}$	24.77 ± 3.55$^{bcd.A}$
20	28.58 ± 3.60$^{a.B}$	27.02 ± 5.30$^{ab.B}$	27.98 ± 3.40$^{a.B}$	29.10 ± 4.06$^{bcd.B}$	21.94 ± 3.58$^{a.A}$
25	28.84 ± 4.56$^{a.C}$	28.84 ± 3.62$^{bc.C}$	27.98 ± 4.53$^{ab.B}$	27.10 ± 3.97$^{ab.B}$	24.45 ± 5.73$^{bcd.A}$
30	29.64 ± 4.24$^{bc.B}$	30.67 ± 3.90$^{c.B}$	29.17 ± 4.41$^{ab.B}$	26.40 ± 6.10$^{a.A}$	25.46 ± 4.54$^{bcd.A}$
35	30.05 ± 4.17$^{bcd.C}$	26.13 ± 2.69$^{a.B}$	28.63 ± 4.43$^{ab.B}$	27.27 ± 4.72$^{ab.B}$	22.18 ± 4.41$^{a.A}$
40	30.21 ± 4.35$^{bcd.C}$	29.91 ± 3.86$^{bc.C}$	27.50 ± 3.35$^{a.BC}$	26.16 ± 4.52$^{a.B}$	21.79 ± 4.62$^{a.A}$

Note: The ultrasound energy density was 20 W/L at different temperatures for each ultrasound time; Data were expressed in the mean ± standard deviation of twenty independent replicates; Different superscript uppercase letters in the same row indicate a significant difference among ultrasound temperature at $p < 0.05$. Different superscript lowercase letters in the same column mean a significant difference among ultrasound time at $p < 0.05$.

$$E(\%) = \frac{1}{N}\sum_{i=1}^{N}\left(\frac{V_e - V_p}{V_e}\right) \times 100 \quad (4)$$

where V_e and V_P are the experimental and predictive values, respectively; N is the number of times the experiment was repeated.

2.8. Pectin determination

Pectin extraction was performed according to Chen, Zhou, He, Liu, Lai, Yang [27]. Fruit flesh (10 g) was boiled for 20 min in 200 mL of ethanol (80%, v/v) with distillation tube, cooled, and then filtered. The residue was treated two more times as above. The residue was then placed in 50 mL of dimethyl sulfoxide (DMSO) and water (9:1, v/v) for 12 h at 4 °C. Subsequently, the mixture was filtered and transferred to 200 mL of chloroform and ethanol (2:1, v/v) for 10 min at 25 °C, filtered, and washed with 200 mL acetone until it was white. The residue was collected as the cell wall material. The cell wall material was put into distilled water (10 mL), agitated for 4 h at 25 °C, and centrifuged at 10,000 × *g* for 10 min at 4 °C. The residue was re-extracted two times. All the supernatants were collected and combined as water-soluble pectin (WSP). Residues were resuspended in 10 mL 50 mmol/L cyclohexanetrans-1,2-diamine tetra-acetate (CDTA, Sinopharm Chemical Reagent, China), shaken for 4 h at 25 °C, and centrifuged as described above. The residue was re-extracted twice with CDTA. All supernatants were collected as chelate-soluble pectin (CSP). Finally, residues were resuspended in 50 mmol/L sodium carbonate solution containing 2 mmol/L CDTA (10 mL), shaken for 4 h at 25 °C, and centrifuged as described above. The procedure was repeated two more times, and the supernatants were collected as sodium carbonate-soluble pectin (SSP).

The pectin content was determined by the colorimetric method according to Filisetti-Cozzi, Carpita and Su, Li, Quek, Huang, Yuan, Li, Shan with some modification [28,29]. Galacturonic acid (Sigma-Aldrich Co., Ltd., St. Louis, MO, USA) was used as the standard. The pectin solution (2 mL) was added into cooled sulphuric acid (12 mL, 98%, w/w) and placed in a test tube. The above mixture was heated in boiled water for 10 min and cooled with running tap water immediately. The carbazole solution (0.5 mL) was mixed into the pectin solution and incubated at 25 °C for 30 min. The absorbance at 530 nm was then measured using a spectrophotometer (722S, Shanghai Instrument Analysis Instrument Co., Ltd., Shanghai, China). Each experiment was performed in triplicate.

2.9. AFM analysis of pectin

Atomic force microscopy was a frequently used instrument for observation of the structure at a nanometer scale. AFM analysis of pectin was performed according to a previous study [9]. Pectin solution (10 mg/L, 10 μL) was pipetted onto a freshly cleaved mica surface. The mica was pasted onto a metal plate and fixed on the sample stage for atomic force microscopy (AFM) (D–5A, Zhuolun MicroNano Equipment Co., Ltd, Shanghai, China). The images were collected using a Si_3N_4 cantilevered probe (MikroMasch, Estonia) in non-contact mode. The resonance frequency of the tip was 20–40 kHz, and the scan rate was about 0.5–2 Hz. The images were analysed offline using the manufacturer's software. The length and width of 200 pectin chains were assessed. The percent of pectin chains of particular width or length among all the chains observed was recorded as the frequency (F_q). More than 20 images were obtained for each sample to obtain credible statistical results.

2.10. Statistical analysis

Each experiment was operated in triplicate. The data were analysed using Excel 2010 (Microsoft Co., Ltd., Washington, USA), SPSS 20 (IBM Co., Ltd., New York, USA), and GraphPad Prism 5 (GraphPad Software Co., Ltd., San Diego, USA) software. The results of the data analysis were expressed in the mean ± standard deviation. Duncan's multiple range test was used to analyse the significant differences. Comparisons that yielded $p < 0.05$ were considered statistically significant.

3. Results and discussions

3.1. Firmness

The effect of ultrasound combined with calcium lactate (U + Ca) on the firmness of cherry tomatoes is shown in Table 1. There were decreasing trend of firmness with increasing ultrasound time for all temperatures except for 15 °C. A significant loss in firmness with increasing time was also observed in the process of treating strawberries with ultrasound [13]. Furthermore, no significant difference in firmness was observed when the ultrasound temperature was 15 °C and the fruit were exposed for 0–15 min. This phenomenon could be explained by the increase in the calcium (Ca) content under U + Ca treatment [12]. There were no obvious changes in firmness of cherry tomatoes among temperature of 20, 25, and 30 °C at different ultrasound time (5–40 min) ($p > 0.05$). Compared with other temperatures, the firmness significantly decreased at 35 °C ($p < 0.05$). The change in firmness is relate to the detrimental effect of high treatment temperature on the cell wall structure. Cell membrane permeability was changed because of structure of fruit tissue change at high temperature, leading to significant water lose [30].

U + Ca treatment might have a positive effect on firmness retention in cherry tomatoes if delivered for a suitable period (15 °C, 15 min). However, as the length of exposure time to ultrasound increased, the structure and stability of the cell wall and pectin chains might be broken down by ultrasound, inducing a series of mechanical and

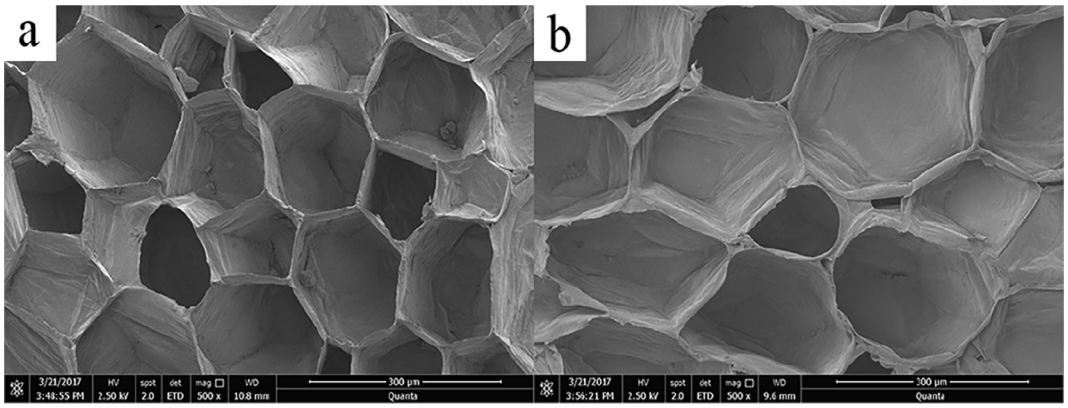

Fig. 1. Effect of ultrasound combined with calcium lactate treatment on the micro structure of cherry tomato observed with SEM: (a) view of tissue in fresh fruit; (b) view of tissue in fruit treated by ultrasound with calcium lactate (20 W/L, 15 min, 15 °C); The micrographs were taken at 500 × magnification (bar = 300 µm).

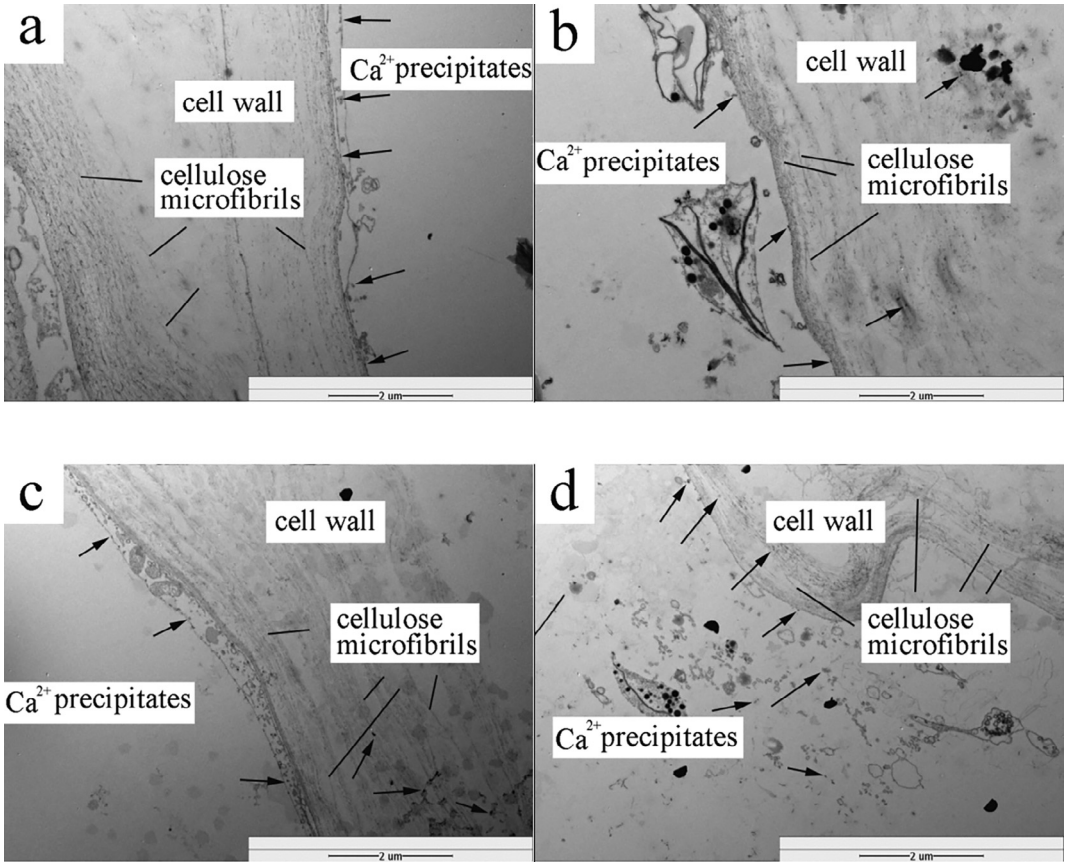

Fig. 2. Effect of ultrasound combined with calcium lactate treatment on distribution of Ca in cherry tomato: (a) the pulp tissue of fresh fruit; (b) the pulp tissue of fruit by ultrasound combined with calcium treatment at 20 W/L for 5 min at 15 °C; (c) the pulp tissue of fruit by ultrasound combined with calcium treatment at 20 W/L for 15 min at 15 °C; (d) the pulp tissue of fruit by ultrasound combined with calcium treatment at 20 W/L for 25 min at 15 °C; The micrographs were taken at 15,000 × magnification (bar = 2 µm).

chemical effects, leading to decreased firmness and accelerated water loss [13].

3.2. Microstructure of tissue in cherry tomato

To better evaluate the effect of ultrasound combined with calcium lactate treatment on the cell wall of cherry tomatoes, SEM was applied to observe the cell wall structure. The cell wall of fresh cherry tomatoes (Fig. 1a) consisted of small cells without intercellular spaces. The boundary and plump stereoscopic structure of cell was observed [22]. In the fruit treated under ultrasound combined with calcium lactate (20 W/L, 15 min, and 15 °C), the cell morphological and structural was similarity to fresh fruit. Smaller intercellular spaces in the cell wall could be seen in treated fruit (Fig. 1b).

But the boundary and plump stereoscopic structure was still intact. Therefore, ultrasound can be used as the pre-treatment of cherry tomatoes for storage, and it does not damage the cell wall structure.

3.3. Distribution of Ca^{2+} in pulp tissue

The distribution of Ca^{2+} in cherry tomatoes during U + Ca treatment is shown in Fig. 2. Ca^{2+} and OsO_4 reacted generating sediment,

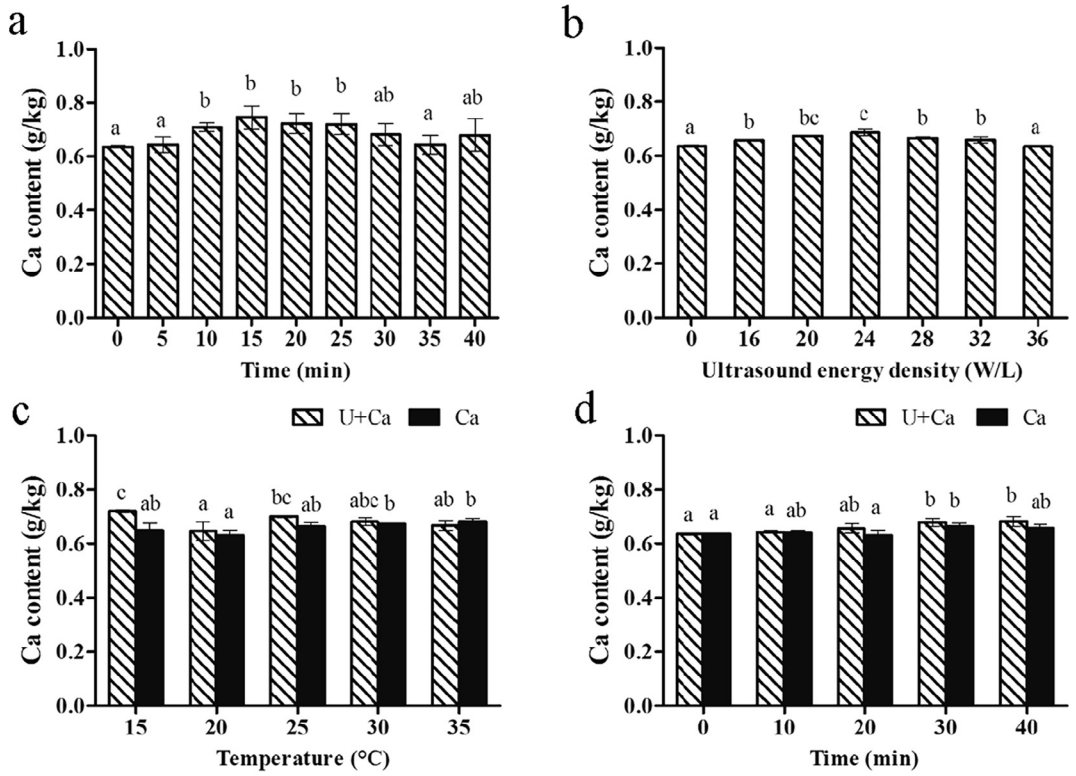

Fig. 3. Effect of ultrasound combined with calcium lactate treatment on the calcium content in cherry tomatoes. Calcium (Ca) content was expressed in grams per kilogram of dry matter. a: calcium content in cherry tomatoes of the ultrasound combined with calcium lactate treated group for different time (20 W/L, 15 °C); b: calcium content in cherry tomatoes of the ultrasound combined with calcium lactate treated group in different ultrasound energy density (20 °C, 20 min); c: calcium content in cherry tomatoes of the ultrasound combined with calcium lactate treated group at different temperature (20 W/L, 20 min); d: calcium content in cherry tomatoes of the ultrasound combined with calcium lactate treated group for different time (20 W/L, 20 °C). Different small case letters indicate a significant difference at $p < 0.05$ within a group.

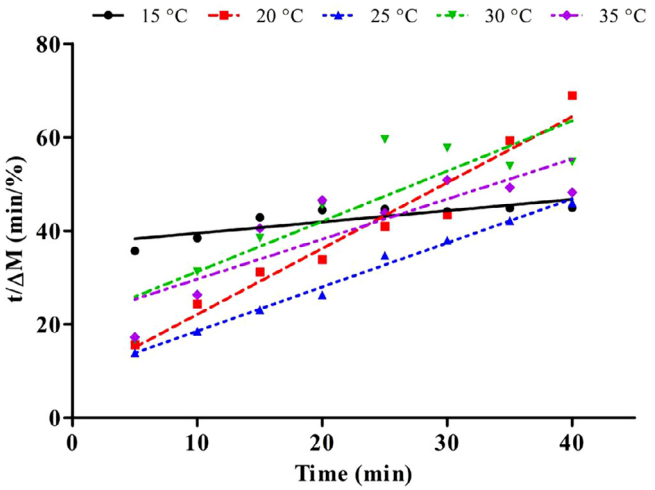

Fig. 4. The simulation curves of solid gain in cherry tomatoes treated by ultrasound combined with calcium lactate at the temperature of 15–35 °C.

and which reflected in black particle form in TEM imagine. In fresh fruit, there was only a little Ca^{2+} distributing on the surface of cell wall, this was the endogenous Ca^{2+} existing the tissue of cherry tomato. After treated for 5 min, some Ca^{2+} distributing in the cell wall and some small pieces on the surface of the cell wall, which may include a small amount of exogenous Ca^{2+}. Then, more black segments in the cell wall were observed in cherry tomatoes treated for 15 min. When cherry tomato was treated by U + Ca for 25 min, more Ca^{2+} distributed on the surface of the cell wall. With increasing of ultrasound time, the Ca^{2+} distributed on the surface of the cell wall increased.

Table 2
The regression coefficients and statistical parameters of Peleg's model.

Temperature (°C)	Regression equation	Statistical parameters		
		R^2	RMSE	E (%)
15	y = 0.2396x + 37.1153	0.7080	1.5410	2.8341
20	y = 1.4091x + 8.0364	0.9579	3.3858	7.1859
25	y = 0.9427x + 9.1157	0.9916	0.9967	2.1248
30	y = 1.0752x + 20.5171	0.7683	6.7642	15.0586
35	y = 0.8567x + 21.0746	0.7461	5.7264	15.4271

In addition, in cherry tomato cell wall, cellulose microfibrils were arranged in parallel. After treatment, the structure of cell wall was not destroyed. So in this ultrasound condition, there was no negative effect on cell wall structure of cherry tomato.

3.4. Calcium content

The calcium content in cherry tomatoes treated with ultrasound combined with calcium lactate is shown in Fig. 3. Compared to fresh fruit, the calcium content in cherry tomato treated under U + Ca increased significantly (Fig. 3a). After treated for 10 min, the calcium content change trend reached to a balance. The Ca content at 15 min was 0.75 g/kg, which was significantly higher than that in fresh fruit (0.64 g/kg, ultrasound for 0 min) ($p < 0.05$). The highest Ca content (0.69 g/kg) was found at an energy density of 24 W/L (Fig. 3b). Fig. 3c showed that the Ca content decreased gradually as the ultrasound temperature increased, with the highest Ca content (0.72 g/kg) being observed at 15 °C. And at this ultrasound temperature, the firmness of

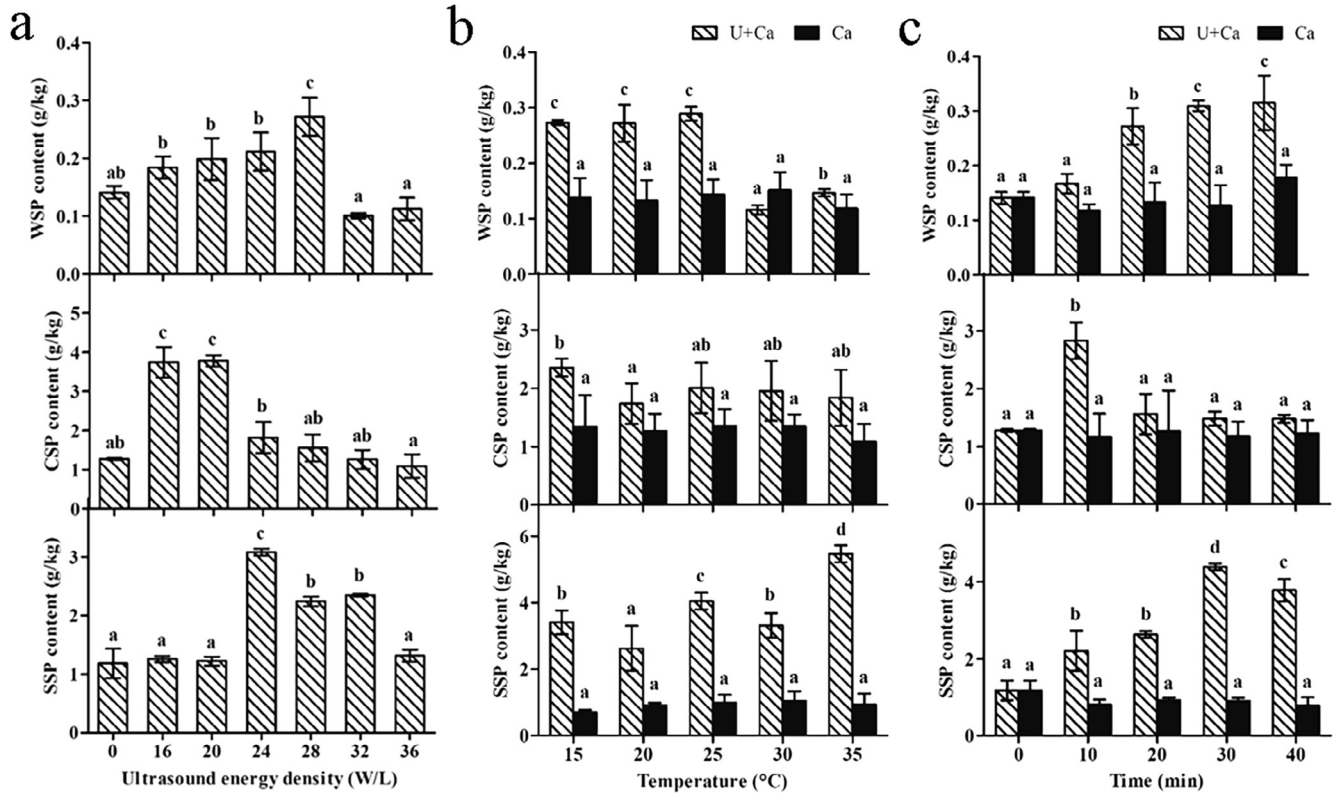

Fig. 5. Effect of ultrasound combined with calcium lactate treatment on water-soluble pectin (WSP), chelate-soluble pectin (CSP), and sodium carbonate-soluble pectin (SSP) contents in cherry tomatoes. a: Pectin from cherry tomatoes of the ultrasound combined with calcium lactate treated group in different ultrasound energy density (20 °C, 20 min); b: Pectin from cherry tomatoes of the ultrasound combined with calcium lactate treated group at different temperature (20 W/L, 20 min); c: Pectin from cherry tomatoes of the ultrasound combined with calcium lactate treated group for different time (20 W/L, 20 °C); Error bars represent the standard deviation of the mean of three replicates. Different small case letters indicate a significant difference at $p < 0.05$ within a group.

cherry tomatoes was maintained well than other temperatures (Table 1). This result indicated that the bidirectionally osmotic exchange effect of Ca was influenced markedly by the ultrasound temperature.

Effects of ultrasound temperature and time on the Ca content of fruit treated with U + Ca and Ca alone were also observed, and the results were shown in Fig. 3c and d. The Ca content of cherry tomatoes in U + Ca treated group was higher (11.10%) than that in Ca treated group at 15 °C. Meanwhile, the result indicated that Ca permeation was promoted by ultrasound. The same result was obtained in jujube fruit treated with ultrasound combined with calcium chloride [12]. Pieczywek, Kozioł, Konopacka, Cybulska, Zdunek [31] reported that the cell wall permeability increased, and the transport of water and solute were facilitated under ultrasound. In view of the change of calcium content, the mass transfer in cherry tomatoes at different ultrasound temperatures was studied.

3.5. Solid gain

The trend of solid gain change was simulated with Peleg's model. The simulation curve and regression equation, and the statistical parameters of Peleg's model, are shown in Fig. 4 and Table 2, respectively. The initial rate of mass transfer was the highest at 20 °C, and it was the lowest at 15 °C according to the intercept value (Fig. 4). In addition, compared with other temperatures, the slope value was the lowest at 15 °C. This result indicated that the solid gain at balanced state was highest at 15 °C.

At low ultrasound temperatures (15 °C, 20 °C and 25 °C), the R^2 values were 0.7080, 0.9579, and 0.9916, respectively. Meanwhile, the RMSE values (1.5410, 3.3858, and 0.9967) and the E value (2.8341%, 7.1859%, and 2.1248%) of 15 °C, 20 °C and 25 °C were lower than that of 30 °C and 35 °C (RMSE: 6.7642 and 5.7264; E: 15.0586% and 15.4271%)(Table 2). However, an E value less than 10% indicated that the fitting degree of this model was acceptable. Thus, the mathematical model could be used in our experiment at 15 °C, 20 °C and 25 °C.

A previous study reported that the mass transfer was enhanced using ultrasound [32]. The microstructure of fruit tissue is changed by the mechanical and chemical effects of ultrasound, leading to the formation of many microscopic channels, which have positive effect on mass transfer [33]. However, the cell wall structure became loose with the increasing of ultrasound temperature. Therefore, Peleg's model cannot be used to describe the solid gain at higher ultrasound temperatures (30 and 35 °C).

3.6. Pectin content

Pectin content is an important quality index of fruit. The content and forms of pectin plays an important role in maintaining the firmness of fruit [34–36]. So the study of pectin content change in different ultrasound condition to indicate the relationship between firmness and pectin. The content of WSP, CSP, and SSP in cherry tomatoes is shown in Fig. 5. The contents of WSP, CSP, and SSP in fresh fruit were 0.14, 1.27, and 1.18 g/kg, respectively. There was no obvious effect of treatment temperature and time on the contents of WSP, CSP, and SSP in cherry tomatoes treated with calcium lactate only. For U + Ca group, ultrasound energy density, temperature and time have significant effects on pectin contents ($p < 0.05$). The maximal values of WSP (0.27 g/kg), CSP (3.78 g/kg), and SSP (3.08 g/kg) were arrived at ultrasound energy density 28, 16 and 20, and 24 W/L, respectively (Fig. 5a). Then, the contents of WSP, CSP, and SSP decreased with further increasing of ultrasound energy density. Aday, Temizkan, Büyükcan, Caner reported that higher ultrasound energy density had a

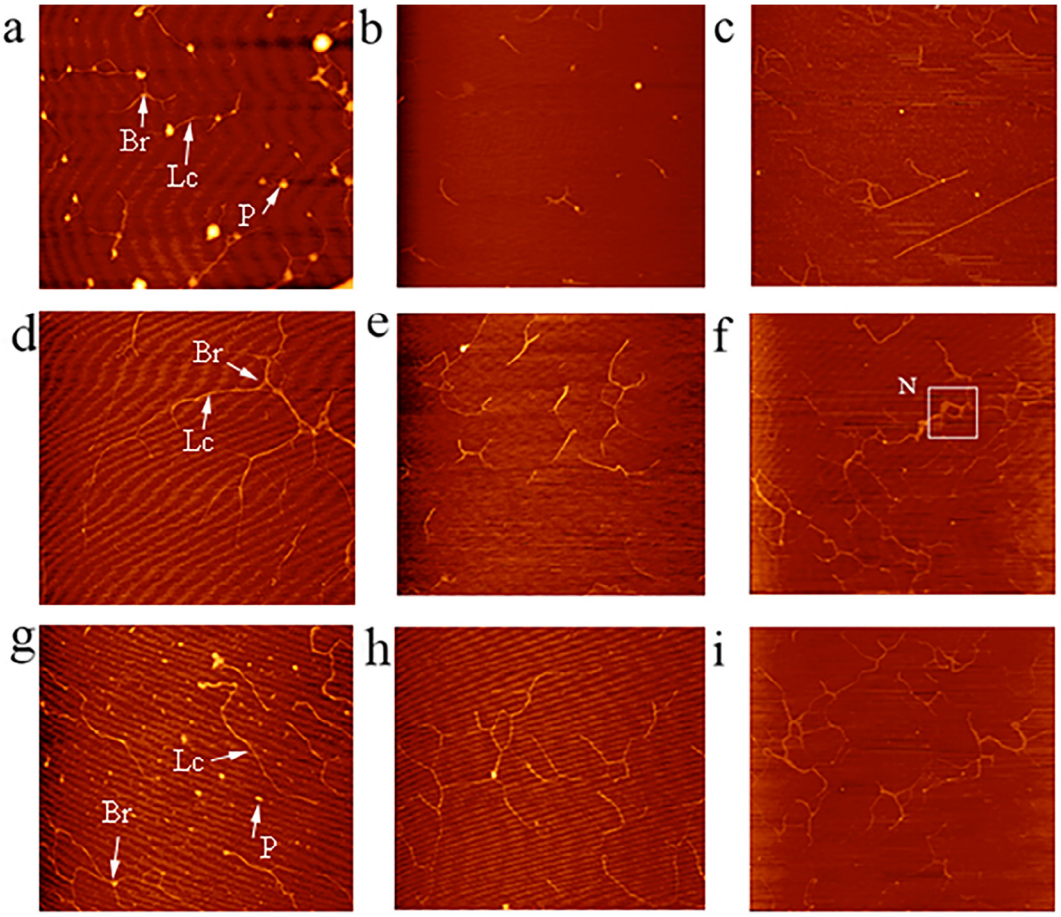

Fig. 6. Atomic force microscopy (AFM) images of water-soluble pectin (WSP) (images a–c), chelate-soluble pectin (CSP) (images d–f), and sodium carbonate-soluble pectin (SSP) (images g–i) chains in cherry tomatoes. a, d, g: images from cherry tomatoes of fresh fruit; b, e, h: images from cherry tomatoes of the calcium lactate treated group (15 min, and 15 °C); c, f, i: images from cherry tomatoes of the ultrasound combined with calcium lactate treated group (20 W/L, 15 min, and 15 °C); Scan area: 5.000 μm × 5.000 μm. Lc: long straight chains; Sc: short chains; Br: branched chains; P: polymer structure; N: net-like structure.

Table 3
Effect of ultrasound combined with calcium lactate treatment on the width (W) and length (L) distribution of pectin chains in cherry tomatoes.

	W (nm)	F_q (%)			L (μm)	F_q (%)		
		Fresh	Ca	U + Ca		Fresh	Ca	U + Ca
WSP	< 20	11.99 ± 1.00^a	12.10 ± 4.00^a	11.90 ± 1.15^a	0–1	56.25 ± 1.25^b	52.50 ± 5.21^b	20.00 ± 7.50^a
	20–40	78.51 ± 5.03^a	80.00 ± 7.01^a	87.00 ± 3.05^b	1–2	36.25 ± 8.75^a	42.50 ± 7.68^b	55.00 ± 7.50^c
	40–60	10.18 ± 5.03^c	7.90 ± 2.00^b	1.09 ± 0.31^a	2–3	6.25 ± 2.25^a	5.00 ± 3.53^a	23.75 ± 1.25^b
	≥60	0.32 ± 0.31^b	0.00 ± 0.00^a	0.00 ± 0.00^a	≥3	1.25 ± 0.25^b	0.00 ± 0.00^a	1.25 ± 0.25^b
CSP	< 20	6.22 ± 0.80^b	3.50 ± 1.20^a	3.48 ± 1.90^a	0–1	1.25 ± 0.25^a	3.00 ± 1.00^b	3.58 ± 1.52^b
	20–40	56.79 ± 4.73^a	55.50 ± 5.80^a	57.00 ± 4.04^a	1–2	37.50 ± 2.50^c	25.00 ± 7.14^a	28.79 ± 4.56^b
	40–60	28.49 ± 4.58^a	30.50 ± 3.20^b	27.04 ± 1.73^a	2–3	45.00 ± 2.50^a	46.70 ± 4.14^a	45.00 ± 4.56^a
	≥60	8.50 ± 5.28^a	10.50 ± 3.50^b	12.48 ± 2.04^c	≥3	16.25 ± 3.75^a	25.30 ± 2.82^b	22.64 ± 1.52^b
SSP	< 20	8.84 ± 3.06^a	14.67 ± 5.78^b	6.40 ± 1.79^a	0–1	12.50 ± 2.50^a	13.35 ± 4.04^a	12.50 ± 2.50^a
	20–40	83.49 ± 4.00^a	76.00 ± 4.00^a	78.40 ± 3.04^a	1–2	57.50 ± 5.48^b	65.35 ± 6.47^c	50.00 ± 2.50^a
	40–60	7.73 ± 4.16^a	8.67 ± 4.02^a	15.20 ± 4.51^b	2–3	20.00 ± 7.50^a	22.30 ± 7.07^a	27.50 ± 2.50^b
	≥60	0.00 ± 0.00^a	2.00 ± 0.08^b	0.00 ± 0.00^a	≥3	1.00 ± 0.50^a	0.00 ± 0.00^a	10.0 ± 2.00^b

Note: F_q: frequency; Error bars represent the standard deviation of the mean of three replicates; Different lowercase letters indicate a significant difference at $p < 0.05$ among the groups.

detrimental effect on cell wall components in cherry tomatoes [37].

The effect of ultrasound temperature on pectin content is shown in Fig. 5b. A significant decrease of WSP content was observed when ultrasound temperature above 25 °C ($p < 0.05$). Ultrasound treatment temperature had no significant effect on the content of CSP ($p > 0.05$). The stability of CSP was increased by the formation of a bridge between Ca^{2+} and CSP, and the increase in cell wall stability, which mediated the effects of ultrasound. A previous study also reported that Ca contributes to inhibit the degradation of CSP in fruit [8]. The SSP content was affect by ultrasound temperature significantly. The lowest and peak SSP content was found at 20 °C, and 35 °C, respectively.

The WSP content increased as a function of ultrasound time (Fig. 5c). The CSP (2.82 g/kg) and SSP contents (4.39 g/kg) peaked at 10 min and 30 min, respectively. The side chains of CSP and SSP

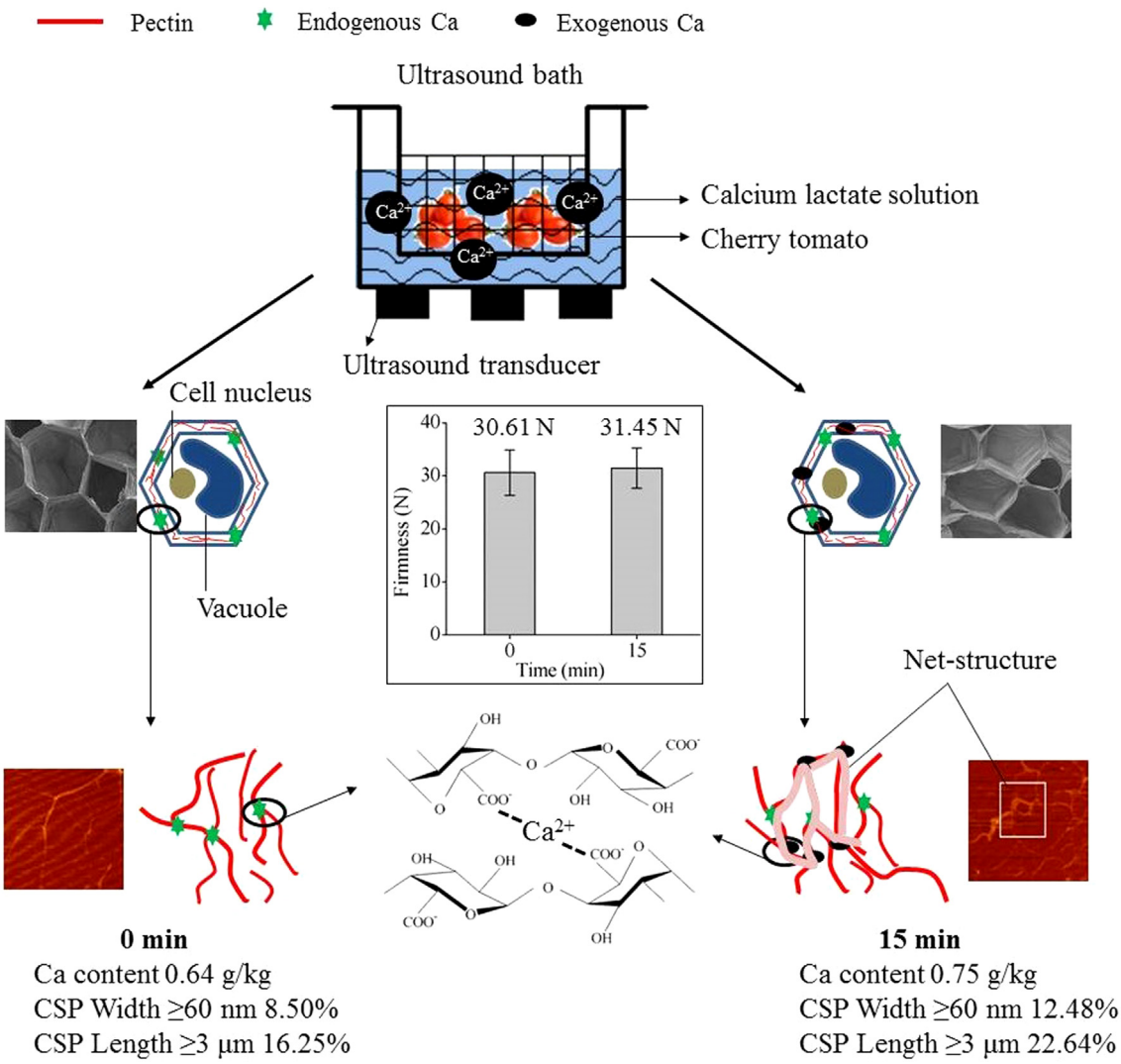

Fig. 7. Schematic image of calcium permeation and pectin chains of cherry tomatoes treated by ultrasound combined with calcium lactate.

disrupted and transformed to WSP by long period of ultrasound treatment [38,39]. Meanwhile, at longer ultrasound treated time and higher temperatures, the tissues of cherry tomato were damaged and the cell wall structure decomposed, resulting in higher levels of SSP dissolved in the extract [40]. In addition, the increasing contents of WSP, CSP, and SSP also resulted from the disassembly of the polysaccharide network and the increase of extraction efficiency by ultrasound [31,41]. Thus, desirable ultrasound conditions should be selected to promote the permeating of Ca^{2+} into fruit, and to maintain the quality properties of fruit.

3.7. AFM analysis of pectin

Pectin is a complex heteropolysaccharide including linear fragments and ramified regions covalently connected [42]. The structure of pectin was changed during ultrasound combined with calcium lactate treatment. The effects of U + Ca and Ca treatment on the AFM images of WSP, CSP, and SSP in cherry tomatoes are shown in Fig. 6. Long chains (Lc), polymers (P), and branched structures (Br) of WSP, CSP and SSP molecules were observed in fresh cherry tomatoes (Fig. 6 a, d, and g) [43]. Only short straight chains, smaller polymers of WSP were observed in Ca treated cherry tomatoes (Fig. 6 b). Long chains, and branched structures of CSP and SSP were observed in Ca treated cherry tomatoes (Fig. 6 e, and h). Meanwhile, long chains, and branched structures were also observed in cherry tomatoes treated with U + Ca at an ultrasound energy density of 20 W/L for 15 min at 15 °C (Fig. 6 c, f, and i).

Notably, Net-like structures (N) were observed in CSP and SSP molecules of cherry tomatoes treated with Ca and U + Ca (Fig. 6e, f, h, i). The net-like structure might be formed by Ca cross-linking with pectin, which would promote the maintenance of firmness. Ultrasound enhanced the permeation of Ca, leading to the formation of a more net-like structure. Thus, ultrasound combined with calcium lactate treatment had positive effect on delaying the reduction in firmness of cherry tomatoes [12].

The width and length values of WSP, CSP, and SSP molecules in cherry tomatoes are shown in Table 3. The width of the WSP molecules in cherry tomatoes was mostly distributed from 20 to 60 nm. In fresh fruit, the width of the WSP molecules was mostly in the range of 20–60 nm at an F_q of 88.69%. No significant increase in the F_q of the width of WSP molecules in cherry tomatoes treated by Ca and U + Ca was found in the range of 20–60 nm. The length of WSP for all groups was mainly in the range of 0–2 μm. After U + Ca treatment, the F_q of WSP with length between 2 and 3 μm increased from 6.25% to 23.75%.

For CSP, the chain width was mostly distributed in the range of 20–40 nm. Increasing phenomena in the F_q of the CSP chain width (20–60 nm) in cherry tomatoes treated with Ca and U + Ca compared with fresh fruit was observed. In fresh fruit, the F_q of the CSP chain

width of more than 60 nm was 8.50%, and that of CSP chains treated with U + Ca was 12.48%. About 70.00% of the pectin length values of treated fruit were greater than 2 μm, which was higher than that in fresh fruit (61.25%). Ultrasound can be used as an effective means to promote Ca permeation of fruit tissue, promoting the formation of Ca^{2+} bridges between pectin chains. The Ca^{2+} bridges between CSP chains were increased by external Ca supplementation, leading to increased pectin stability [8]. There was no significant difference among SSP of all groups for width in the range of 20–40 nm. Larger F_q of width (≥40 nm) and length (≥2 μm) of SSP chains was observed in U + Ca treated group.

A schematic image of the calcium permeation and proposed pectin changes in cherry tomato treated with U + Ca is shown in Fig. 7. The firmness could be maintained and Ca content increased in the cherry tomatoes under suitable ultrasound conditions. And the cell wall of cherry tomato was not destroyed. Meantime, U + Ca treatment can promote the calcium permeating into the tissue, increasing the calcium distribution in cell wall of cherry tomato (Table 1 and Fig. 2 and Fig. 3). In addition, Ca^{2+} in fruit tissue combines with non-esterified C-6 in galacturonic acid residues to form a stable composite structure [44], leading to pectin stability increase. The width and length values of CSP and SSP in cherry tomatoes treated with U + Ca were higher than those in fresh and Ca treated fruit (Table 3). Net-structure formation between Ca^{2+} and CSP inhibited the pectin substance dissolution, which contributed to cherry tomato firmness. More Ca^{2+} permeated into the tissue of cherry tomato under U + Ca treatment, and more stable net-structure was formed. In conclusion, U + Ca treatment can as a promising method to improve the texture of cherry tomatoes at suitable condition.

4. Conclusions

This study demonstrated the effect of ultrasound combined with calcium lactate treatment on the firmness, Ca permeation and pectin properties of cherry tomatoes. The firmness of cherry tomatoes decreased with increasing ultrasound temperature and time. The firmness (31.45 N) of cherry tomatoes was maintained when ultrasound energy density was 20 W/L for 15 min at 15 °C. The Ca content increased in treated fruit and its permeation was affected significantly by ultrasound factors. Meanwhile, the Peleg's model could be used to express the change of solid gain in cherry tomatoes under ultrasound treatment at 15, 20, and 25 °C. The contents of WSP, CSP, and SSP in cherry tomatoes may be increased under suitable ultrasound energy intensity, temperature and time. According to AFM results, a higher F_q for the width (≥40 nm) and length (≥2 μm) for CSP and SSP chains was observed in treated groups than that in fresh fruit. Ultrasound combined with calcium lactate treatment promoted the crosslinking of Ca^{2+} and CSP. The results suggested that ultrasound combined with calcium lactate treatment is a promising technique to maintain the texture of cherry tomatoes. The future work should be carried out to investigate its effects on the shelf-life and consumer acceptability of perishable fruit.

Acknowledgements

Projects 31501535, 31371851 and 31071617 supported by NSFC and Foundation for Young Key Teachers of Henan University of Technology (21420051). The Singapore Ministry of Education Academic Research Fund Tier 1 (R-143-000-A40-114) and Natural Science Foundation of Jiangsu Province (BK20181184) also contributed to this research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ultsonch.2019.104784.

References

[1] P. Flores, E. Sánchez, J. Fenoll, P. Hellín, Genotypic variability of carotenoids in traditional tomato cultivars, Food Res. Int. 100 (2017) 510–516.
[2] L.C. Sow, F. Tirtawinata, H. Yang, Q. Shao, S. Wang, Carvacrol nanoemulsion combined with acid electrolysed water to inactivate bacteria, yeast *in vitro* and native microflora on shredded cabbages, Food Control 76 (2017) 88–95.
[3] J.S. Won, S.J. Lee, H.H. Park, K.B. Song, S.C. Min, Edible coating using a chitosan-based colloid incorporating grapefruit seed extract for cherry tomato safety and preservation, J. Food Sci. 83 (2018) 138–146.
[4] M.M.C. Sobral, C. Nunes, A. Maia, P. Ferreira, M.A. Coimbra, Conditions for producing long shelf life fruit salads processed using mild pasteurization, LWT-Food Sci. Technol. 85 (2017) 316–323.
[5] C. Liu, H. Zheng, K. Sheng, W. Liu, L. Zheng, Effects of postharvest UV-C irradiation on phenolic acids, flavonoids, and key phenylpropanoid pathway genes in tomato fruit, Sci. Hortic. 241 (2018) 107–114.
[6] E. Aguayo, C. Requejo-Jackman, R. Stanley, A. Woolf, Hot water treatment in combination with calcium ascorbate dips increases bioactive compounds and helps to maintain fresh-cut apple quality, Postharvest Biol. Technol. 110 (2015) 158–165.
[7] I. Ali, N.A. Abbasi, I.A. Hafiz, Physiological response and quality attributes of peach fruit cv. florida king as affected by different treatments of calcium chloride, putrescine and salicylic acid, Pak. J. Agr. Sci. 51 (2014) 33–39.
[8] H. Yang, Q. Wu, L.Y. Ng, S. Wang, Effects of vacuum impregnation with calcium lactate and pectin methylesterase on quality attributes and chelate-soluble pectin morphology of fresh-cut papayas, Food Bioprocess Technol. 10 (2017) 901–913.
[9] J. Mao, L. Zhang, F. Chen, S. Lai, B. Yang, H. Yang, Effect of vacuum impregnation combined with calcium lactate on the firmness and polysaccharide morphology of kyoho grapes (*Vitis vinifera* × *V. labrusca*), Food Bioprocess Technol. 10 (2017) 699–709.
[10] S. Chakraborty, D. Baier, D. Knorr, H.N. Mishra, High pressure inactivation of polygalacturonase, pectinmethylesterase and polyphenoloxidase in strawberry puree mixed with sugar, Food Bioprod. Process. 95 (2015) 281–291.
[11] J.X. Chong, S. Lai, H. Yang, Chitosan combined with calcium chloride impacts fresh-cut honeydew melon by stabilising nanostructures of sodium-carbonate-soluble pectin, Food Control 53 (2015) 195–205.
[12] H. Zhi, Q. Liu, J. Xu, Y. Dong, M. Liu, W. Zong, Ultrasound enhances calcium absorption of jujube fruit by regulating the cellular calcium distribution and metabolism of cell wall polysaccharides, J. Sci. Food Agr. 97 (2017) 5202–5210.
[13] S. Cao, Z. Hu, B. Pang, H. Wang, H. Xie, W. Feng, Effect of ultrasound treatment on fruit decay and quality maintenance in strawberry after harvest, Food Control 21 (2010) 529–532.
[14] J. Pinheiro, C. Alegria, M. Abreu, E.M. Gonçalves, C.L.M. Silva, Influence of postharvest ultrasounds treatments on tomato (*Solanum lycopersicum, cv. Zinac*) quality and microbial load during storage, Ultrason. Sonochem. 27 (2015) 552–559.
[15] F. Chemat, Zill-e-Huma, M.K. Khan, Applications of ultrasound in food technology: Processing, preservation and extraction, Ultrason. Sonochem. 18 (2011) 813–835.
[16] A.L.A. Duarte, D.K.A. do Rosário, S.B.S. Oliveira, H.L.S. de Souza, R.V. de Carvalho, J.C.S. Carneiro, P.I. Silva, P.C. Bernardes, Ultrasound improves antimicrobial effect of sodium dichloroisocyanurate to reduce *Salmonella* Typhimurium on purple cabbage, Int. J. Food Microbiol. 269 (2018) 12–18.
[17] W. Wang, W. Chen, M. Zou, R. Lv, D. Wang, F. Hou, H. Feng, X. Ma, J. Zhong, T. Ding, X. Ye, D. Liu, Applications of power ultrasound in oriented modification and degradation of pectin: a review, J. Food Eng. 234 (2018) 98–107.
[18] L.R. Howerd, R.W. Buescher, Cell wall characteristics and firmness of fresh pack cucumber pickles affected by pasteurization and calcium chloride, J. Food Biochem. 14 (1990) 31–43.
[19] N. Li, F. Chen, F. Cui, W. Sun, J. Zhang, L. Qian, Y. Yang, D. Wu, Y. Dong, J. Jiang, H. Yang, Improved postharvest quality and respiratory activity of straw mushroom (*Volvariella volvacea*) with ultrasound treatment and controlled relative humidity, Sci. Hortic. 225 (2017) 56–64.
[20] L. Zhang, S. Zhao, S. Lai, F. Chen, H. Yang, Combined effects of ultrasound and calcium on the chelate-soluble pectin and quality of strawberries during storage, Carbohydr. Polym. 200 (2018) 427–435.
[21] Y. Xin, F. Chen, S. Lai, H. Yang, Influence of chitosan-based coatings on the physicochemical properties and pectin nanostructure of Chinese cherry, Postharvest Biol. Technol. 133 (2017) 64–71.
[22] L. Wang, P. Jin, J. Wang, L. Jiang, T. Shan, Y. Zheng, Effect of β-aminobutyric acid on cell wall modification and senescence in sweet cherry during storage at 20 °C, Food Chem. 175 (2015) 471–477.
[23] X. Kou, M. Wu, L. Li, S. Wang, Z. Xue, B. Liu, Y. Fei, Effects of $CaCl_2$ dipping and pullulan coating on the development of brown spot on 'Huangguan' pears during cold storage, Postharvest Biol. Technol. 99 (2015) 63–72.
[24] K.A.G. Sotelo, N. Hamid, I. Oey, C. Pook, N. Gutierrez-Maddox, Q. Ma, S.Y. Leong, J. Lu, Red cherries (*Prunus avium* var. Stella) processed by pulsed electric field – physical, chemical and microbiological analyses, Food Chem. 240 (2018) 926–934.
[25] M. Peleg, An empirical model for the description of moisture sorption curves, J. Food Sci. 53 (1988) 1216–1219.
[26] Y. Deng, Y. Zhao, Effects of pulsed-vacuum and ultrasound on the osmodehydration kinetics and microstructure of apples (Fuji), J. Food Eng. 85 (2008) 84–93.
[27] L. Chen, Y. Zhou, Z. He, Q. Liu, S. Lai, H. Yang, Effect of exogenous ATP on the postharvest properties and pectin degradation of mung bean sprouts (*Vigna radiata*), Food Chem. 251 (2018) 9–17.
[28] T.M.C.C. Filisetti-Cozzi, N.C. Carpita, Measurement of uronic acids without interference from neutral sugars, Anal. Biochem. 197 (1991) 157–162.
[29] D. Su, P. Li, S.Y. Quek, Z. Huang, Y. Yuan, G. Li, Y. Shan, Efficient extraction and

characterization of pectin from orange peel by a combined surfactant and microwave assisted process, Food Chem. 286 (2019) 1–7.
[30] D. Salvatori, S.M. Alzamora, Structural changes and mass transfer during glucose infusion of apples as affected by blanching and process variables, Dry. Technol. 18 (2000) 361–382.
[31] P.M. Pieczywek, A. Kozioł, D. Konopacka, J. Cybulska, A. Zdunek, Changes in cell wall stiffness and microstructure in ultrasonically treated apple, J. Food Eng. 197 (2017) 1–8.
[32] A.M.M. Ali, H. Kishimura, S. Benjakul, Physicochemical and molecular properties of gelatin from skin of golden carp (*Probarbus Jullieni*) as influenced by acid pretreatment and prior-ultrasonication, Food Hydrocolloid. 82 (2018) 164–172.
[33] K. Siucińska, D. Konopacka, Application of ultrasound to modify and improve dried fruit and vegetable tissue: a review, Dry. Technol. 32 (2014) 1360–1368.
[34] J.D.J. Ornelas-Paz, B.M. Quintana-Gallegos, P. Escalante-Minakata, Jaime Reyes-Hernández, J.D. Pérez-Martínez, C. Rios-Velasco, S. Ruiz-Cruz, Relationship between the firmness of Golden Delicious apples and the physicochemical characteristics of the fruits and their pectin during development and ripening, J. Food Sci. Technol. 55 (2018) 33–41.
[35] W. Tang, F. Dong, K. Wong, Y. Wang, Preparation, characterization and in vitro release of zein-pectin capsules for target delivery, Curr. Drug Deliv. 12 (2015) 397–405.
[36] N. Zhu, W. Huang, D. Wu, K. Chen, Y. He, Quantitative visualization of pectin distribution maps of peach fruits, Sci. Rep.-uk 7 (2017) 2–10.
[37] M.S. Aday, R. Temizkan, M.B. Büyükcan, C. Caner, An innovative technique for extending shelf life of strawberry: ultrasound, LWT-Food Sci. Technol. 52 (2013) 93–101.
[38] L. Zhang, X. Zhang, D. Liu, T. Ding, X. Ye, Effect of degradation methods on the structural properties of citrus pectin, LWT-Food Sci. Technol. 61 (2015) 630–637.
[39] S.G. Gwanpua, B.E. Verlinden, M.L.A.T.M. Hertog, B.M. Nicolai, M. Hendrickx, A. Geeraerd, A transcriptomics-based kinetic model for enzyme-induced pectin degradation in apple (*Malus* × *domestica*) fruit, Postharvest Biol. Technol. 130 (2017) 64–74.
[40] M. Saeeduddin, M. Abid, S. Jabbar, T. Wu, M.M. Hashim, F.N. Awad, B. Hu, S. Lei, X. Zeng, Quality assessment of pear juice under ultrasound and commercial pasteurization processing conditions, LWT-Food Sci. Technol. 64 (2015) 452–458.
[41] C.H. Su, M.N. Lai, L.T. Ng, Effects of different extraction temperatures on the physicochemical properties of bioactive polysaccharides from Grifola frondosa, Food Chem. 220 (2017) 400–405.
[42] S.S. Ferreira, C.P. Passos, P. Madureira, M. Vilanova, M.A. Coimbra, Structure–function relationships of immunostimulatory polysaccharides: a review, Carbohydr. Polym. 132 (2015) 378–396.
[43] L. Zhang, F. Chen, S. Lai, H. Wang, H. Yang, Impact of a soybean protein isolate-chitosan edible coating on the softening of apricot fruit during storage, LWT-Food Sci. Technol. 96 (2018) 604–611.
[44] M.C. Jarvis, D.C. Apperley, Chain conformation in concentrated pectic gels: evidence from ^{13}C NMR, Carbohyd. Res. 275 (1995) 131–145.