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ORIGINAL PAPER

In vitro study of the interaction between pectinase and chelate-soluble pectin in postharvest apricot fruits

Yamin Chen · Fusheng Chen · Shaojuan Lai · Hongshun Yang · Hui Liu · Kunlun Liu · Guanhao Bu · Yun Deng

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Abstract Firmness is one of the most important quality attributes of apricot fruits and is associated with chelate-soluble pectin (CSP). To elucidate the changes in CSP during postharvest, in vitro investigation of pectinase effects on CSP of apricot fruits was applied to simulate the in vivo changes in CSP fraction during postharvest. It was found that effects of pectinase (40 U/mg) treatment were similar to those of storage time on CSP nanostructures from the results of atomic force microscopy. Relative frequency of widths less than 31.25 nm was 3.6 % for control group, while it was 16.2, 52.0 and 65.5 %, respectively, for 1:10,000 (pectinase/CSP), 1:1,000 and 1:100 pectinase-treated groups. Most of the CSP lengths were 0.5–1 μm, while it was 0–1 μm for pectinase-treated groups. Pectinase treatment had some similar effects on CSP fraction as storage time.

Keywords Nanostructure · Atomic force microscopy (AFM) · Apricot · Pectin · Pectinase

Y. Chen · F. Chen · S. Lai · K. Liu · G. Bu
College of Food Science and Technology, Henan University of Technology, Zhengzhou 450052, Henan, China

H. Yang (✉)
Food Science and Technology Program, Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore
e-mail: chmynghs@nus.edu.sg

H. Liu
Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences, Zhengzhou 450009, Henan, China

Y. Deng
Department of Food Science and Technology, College of Agriculture & Biology, Shanghai Jiao Tong University, 800 Dongchuan Rd., Minhang, Shanghai 200030, China

Introduction

Apricots (*Prunus armeniaca* L.), a kind of climacteric fruits, are favored by consumers for their flavor and texture, but they are highly perishable. Apricots soften rapidly after harvest. Once softened, they are easily damaged such as latent damage, which limits their marketing time [1, 2]. Therefore, a common practice for handling and storing apricots is early harvest and applying postharvest technologies to extend the shelf life [1–4]. The main problem during marketing of apricots is excessive softening. It has been reported that propylene treatment hastens apricots' softening [5], while it is also known that softening may begin before the peak of ethylene production [6]. Therefore, little is known of the mechanism of softening in these fruits, and even the role of ethylene is unclear.

Firmness is a major quality factor affecting the commercial value of apricot fruits. Decreased firmness or softening of fruits was reported to be associated with the depolymerization and solubility of polysaccharides of fruit cell wall [7]. Fruit softening is associated with pectin solubility and sugar loss from cell wall fractions, especially fraction of CSP [8]. Appropriate concentration of calcium can exhibit the textural degradation by connecting ionically bound pectins [3, 4, 9–11]. Appropriate calcium treatment can decrease the loss of CSP and disaggregation of the middle lamella of fruits, thus maintaining the integrity of fruit middle lamella [12].

It is widely accepted that morphology arrangement is critical in determining roles of cell wall materials during postharvest in addition to chemical compositions. However, detailed evolution of fruit CSP is not clear during postharvest considering that what are the major reactions participating in this process is not clear due to the heterogeneous property of the CSP morphology in nanoscale.

Atomic force microscopy (AFM) is a powerful nanotechnology tool to investigate heterogeneous macromolecules [13]. It characterizes variable structures of CSP rather than just providing sample-wide average of the samples as many other methods [13]. Different sources and existing forms of pectins characterized by AFM and similar equipment have been reported, including tomato and beet [14, 15], peaches [16], cherries [17], strawberries [18] and jujubes [19]. These AFM results indicated depolymerization of fruit pectins during fruit ripening. However, to our best knowledge, there was no report about the effects of pectinase on pectin for simulating the changes in pectins during postharvest handling and storage. Using in vitro AFM study would provide an approach to directly study the effects of pectinase on individual pectin molecules and help to elucidate the textural changes in apricot fruits during storage.

The objective of this study was to elucidate the role of pectinase in postharvest quality and property changes in apricot fruits. The nanostructural changes in CSP under pectinase treatment were investigated and compared with natural changes in CSP during storage in vivo. Qualitative and quantitative morphological changes in CSP were determined. The results could help to elucidate the fundamental changes in fruit polysaccharides and textural changes in postharvest fruits during storage.

Materials and methods

Fruits

'Jinhong' apricot (*Prunus armeniaca* L.) fruits were harvested in an orchard in Zhengzhou, Henan Province, China, and transported to the laboratory within 2 h. Only fruits with medium size at firm ripe (mature with change of peel color, about 1 week before fully ripe) of ripening stage with similar color and stalks were chosen for the experiment [20]. The firmness was $9.74 \pm 3.50\ N$ ($n = 10$).

Preparation of cell wall materials and extraction of CSP

Cell wall material (CWM) was prepared according to a previous method [11]. Peeled apricot fruits (10 g) were boiled for 20 min in 80 % ethanol (v/v) and filtered. This procedure was repeated three times, and then the residue was transferred to 50 mL DMSO/H_2O (9:1, v/v) and incubated at 4 °C for 12 h. The solution was filtered, and the solid residue was transferred to chloroform–ethanol solution (2:1, v/v) for 10 min and further treated with acetone after a filtration. The solid residue recovered was CWM.

The CWM from each group was treated with 10 mL ultra-purified water at 25 °C for 4 h for three times, and then it was centrifugated at 10,000×*g* at 4 °C for 10 min (Shanghai Anting Scientific Instrument Factory, Shanghai, China). The solid residue was then treated with 10 mL 50 mM cyclohexane-trans-1, 2-diamine tetra-acetate (CDTA) on a shaker at 25 °C for 4 h. After centrifugation (10,000×*g*) at 4 °C for 10 min, the supernatant was collected. The precipitates were subject to extraction with the above-mentioned conditions for two more times. Then, all the above three supernatants were pooled as fraction of CSP. The solution was stored at −18 °C for further analysis [16].

Pectinase treatment on CSP

Carbazole colorimetry method was applied for quantifying the CSP solutions with galacturonic acid as standard [17]. CSP solution (2 mL) was mixed with 12 mL of sulfuric acid (98 %, w/w) in a test tube and cooled using continuous tap water, and the mixed solution was then boiled for 10 min and cooled again. The solution was added with 0.5 mL carbazole ethanol solution and incubated at room temperature for 30 min. Concentration of the CSP was determined by recording the absorbance at 540 nm using a UV-2000 spectrophotometer (Unico Instrument Co., Ltd, Shanghai, China) with galacturonic acid as standard (Sigma-Aldrich Co., Ltd., St. Louis, MO, USA). The CSP solutions were adjusted to a same concentration and treated with pectinase solution.

CSP extracted from apricot fruits was treated with pectinase (activity 40 U/mg; Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) in phosphate buffer at pH 5.0. The initial CSP was divided into four groups, they were treated with pectinase at different ratios, namely 0, 1:10,000, 1:1,000 and 1:100 (pectinase/CSP, c/c), at 5 °C for 5 min, then they were placed in boiling water for inactivating enzyme 5 min, and finally, the samples were cooled using ice bath [21].

AFM operation and image analysis of CSP fractions

Nanostructures of CSP were characterized using a Vecco multimode NanoScope IIIa AFM (Digital Instruments, C.A., USA) equipped with E (J) scanner according to the previous methods [11, 16]. Diluted solution of 10 μL at approximately 10 μg mL^{-1} was dropped onto the surface of a freshly cleaved mica sheet. A slight molecular combing technique was applied on the solution using a glass coverslip to comb the solution for extending the pectin molecules [16]. The mica with solution was then dried in air at room temperature. After that, tapping mode AFM imaging was performed in air using a NSC 11/no Al tip (MikroMasch, Wilsonville, OR, USA) with a resonance frequency of 330 kHz [16, 22]. The force constant (determined by the dimensions and material) of the cantilever B of the tip was

48 N/m, according to the manufacturer of the AFM tip. The scan rate was set at 0.3–1 Hz.

The AFM images obtained were analyzed off-line with AFM software provided by the company (Version 5.30r3sr3). Section analysis was performed, and the surface profiles of the sections were then plotted to determine the width (*W*, calculated by the peak width of chain half-height) and height (*V*, the height of pectin chains relative to the mica plane surface) of pectin molecules by horizontal and vertical distances, respectively, according to our previous publication [23]. The length (*L*) of single pectin molecule was recorded by plotting the molecule with the software [16]. A number of particular chain widths or lengths observed were set as frequency (Fq). For each sample, more than 10 images were statistically analyzed for getting statistical results.

Statistical analysis

The data were analyzed with SPSS software version 13.0 for Windows (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) was performed for determining the effects of pectinase on nanostructural degradation of CSP molecules. Mean comparisons were conducted using Duncan's test for determining differences between treatments, and comparisons that yielded $P < 0.05$ were considered significant.

Results and discussion

Effect of pectinase treatment on nanostructure of CSP molecules

Low temperature (5 °C) was applied for studying the effects of pectinase on CSP fractions because low temperature could partially retard the enzymatic treatment, considering that enzymatic treatment could be very efficient and quick at room temperature. Thus, the effect of different concentrations of pectinase on pectins could be differentiated from AFM results.

AFM could investigate qualitative and quantitative dimensions including width, length and height of individual CSP chains. Qualitative and quantitative information of CSP pectins after pectinase treatment (pectinase/pectin from 1:10,000 to 1:100) was compared to those without pectinase treatment.

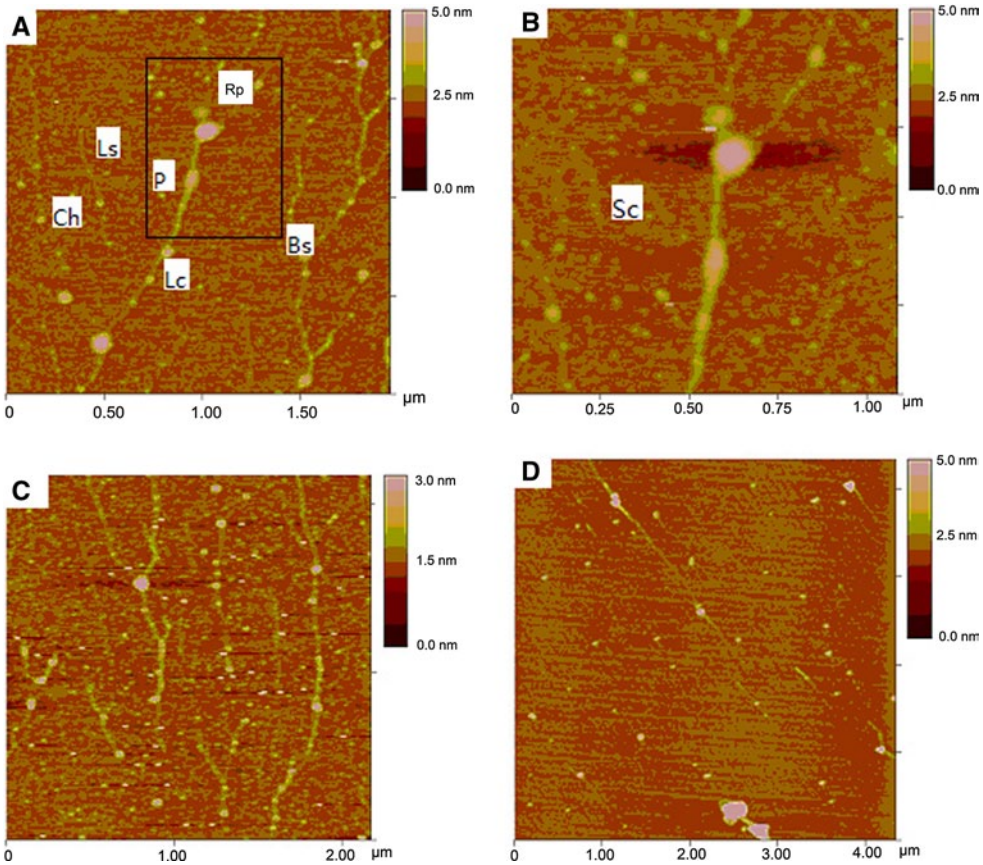

Fig. 1 AFM images of chelate-soluble pectin (CSP) from apricot without pectinase treatment and mild pectinase treatment (1:10,000). **a** Pectins without pectinase treatment, scan area 2.00 μm × 2.00 μm, *height bar* = 5 nm; **b** zoom plane image in the marked region of **a**, respectively, scan area: 1.09 μm × 1.09 μm, *height bar* = 5 nm; **c** another image of pectins without pectinase treatment, scan area 2.17 μm × 2.17 μm, *height bar* = 3 nm; **d** plane images of pectins with mild pectinase treatment (1:10,000, pectinase/CSP), scan area 4.34 μm × 4.34 μm, *height bar* = 5 nm. *Note* Bs: branching structures; Ch = chelator, CDTA; P = polymers; Lc = long chain; Ls = linear single fraction; Rp = releasing point of pectin releasing from the CDTA; Sc: short chain

Qualitative nanostructure

AFM images of CSP from fresh apricot fruits with different concentrations of pectinase treatment are shown in Figs. 1 and 2. The results indicated that CSP chains without pectinase treatment were long (around 2–3 μm) and branched (Fig. 1a), which was similar to CSP of other fruits like yellow peaches [16]. With the increase in pectinase concentrations, higher frequency of short chains (<1,000 nm) and more cleavage points appeared, and polymers of CSP chains reduced.

AFM is appropriate in analyzing heterogeneous structures of CSP, for instance, branching structure (Bs), linear single fraction (Ls), long chain (Lc), short chain (Sc), cleavage point (Cp), releasing point of pectin releasing from CDTA (Rp) and polymer (P) (Fig. 1) [11, 16].

Quantitative nanostructure

Quantitative analysis of CSP morphology includes general and individual structure information analysis [11]. For general information, about 30 % of CSP chains were single or multiple branching structures. A typical branching CSP was represented as 'Bs' in figures (Fig. 1a, for instance). After pectinase treatment (1:10,000), only about 20 % of the CSP chains had branching structures. This is related to reduced-carboxyl-based cross-linking sites, which were destroyed gradually during fruit softening because of action of pectin-degrading enzymes, resulting in deceased branches [24]. Our result was consistent with a previous report [20].

Nanostructural morphology changes in CSP with different pectinase concentrations could be analyzed and demonstrated. Tables 1 and 2, corresponding to the results of Figs. 1 and 2, respectively, show the effects of pectinase treatment on the widths of CSP chains and the corresponding Fq of these chains. The distribution of CSP widths of apricot fruits by pectinase treatment was similar to the effect of storage time on CSP chains [11]. In both situations, CSPs shared many limited and intermittent common values.

As shown in Tables 1 and 2, the CSP widths were much influenced by the pectinase treatment, the Fq of chains of small width increased with pectinase concentration, which was similar to the effects of storage time on CSP [11]. For differently treated groups, 1:100 pectinase-treated group had more Fq of small-width chains than control and 1:10,000 pectinase-treated group. The Fq of widths smaller than 31.25 nm for 1:100 pectinase-treated group was 65.5 %, while it was 3.6, 16.2 and 52.0 % for control, 1:10,000 and 1:1,000 pectinase-treated groups, respectively. Meanwhile, 1: 100 pectinase-treated group had less Fq of large-width chains than control and 1:10,000 pectinase-treated groups, for instance, sum of Fq of widths

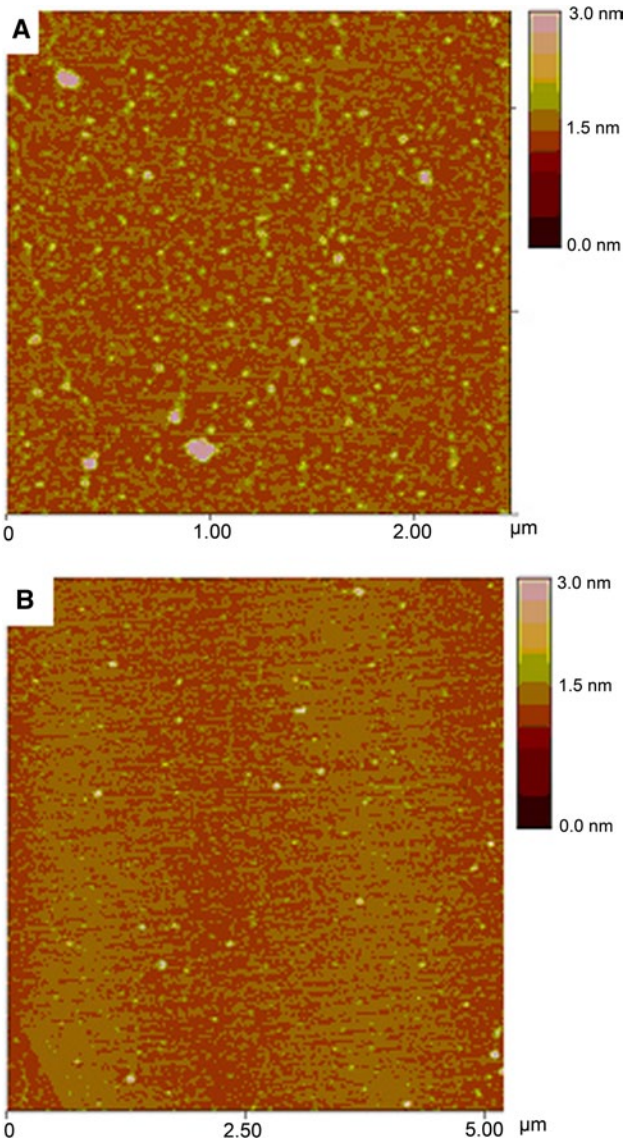

Fig. 2 AFM images of chelate-soluble pectin (CSP) from apricot with significant pectinase treatments. **a** Image of pectins with pectinase treatment (1:1,000, pectinase/CSP), scan area 2.50 μm × 2.50 μm; **b** image of pectins with pectinase treatment (1:100, pectinase/CSP), scan area 5.10 μm × 5.10 μm

larger than 82.03 nm for 1:100 group was 0 %, while it was 32.0, 5.4 and 1.8 % for control, 1:10,000 and 1:1,000 pectinase-treated groups, respectively (Tables 1, 2). Higher-concentration-pectinase (1:100)-treated group decreased the capability of cross-linking of homogalacturonans by enzymatically degrading the CSP molecules.

For apricot CSP heights (V), most chains were between 0.2 and 3.0 nm, especially within 1–2 nm [11]. No significant differences in *V* values were found after pectinase treatment (Tables 1, 2), which indicates that pectinase treatment only affected specific dimensions of CSP such as length and width.

Table 1 Height (*V*) and frequency (Fq) of particular chain width (*W*) of chelate-soluble pectin (CSP) chains without pectinase treatment and mild pectinase treatment (1:10,000)

W (nm)	Without pectinase treatment		Pectinase/CSP (1:10,000)	
	Fq (*N* (%))	*V* (nm)	Fq (*N*(%))	Fq (*N* (%))
11.72	–	–	–	–
15.63	–	–	–	–
19.53	–	–	–	–
23.44	2 (3.6)	0.73 ± 0.28	6 (16.2)	0.22 ± 0.07
27.34	–	–	–	–
31.25	–	–	–	–
35.16	2 (3.6)	0.86 ± 0.21	3 (8.1)	0.41 ± 0.05
39.06	3 (5.4)	0.59 ± 0.07	5 (13.5)	0.54 ± 0.18
42.97	–	–	–	–
46.88	5 (8.9)	0.41 ± 0.03	3 (8.1)	0.48 ± 0.12
58.59	9 (16.1)	1.02 ± 0.40	13 (35.1)	0.66 ± 0.19
65.59	4 (7.1)	1.19 ± 0.51	–	–
70.31	1 (1.8)	1.73 ± 0.00	–	–
78.13	12 (21.4)	1.24 ± 0.40	5 (13.5)	1.16 ± 0.33
93.75	2 (3.6)	1.50 ± 0.03	–	–
97.66	2 (3.6)	1.87 ± 0.35	–	–
117.19	4 (7.1)	1.27 ± 0.43	1 (2.7)	1.20 ± 0.00
156.25	3 (5.4)	1.12 ± 0.39	–	–
175.78	5 (8.9)	1.77 ± 0.98	1 (2.7)	1.63 ± 0.00
234.38	2 (3.6)	1.02 ± 0.30	–	–

Symbol '–' means no detection or below the detection level

W, the peak width of chain half-height; V, the height of pectin chains; Fq, the number of times particular pectin chain widths were observed

Table 2 Height (*V*) and frequency (Fq) of particular chain width (*W*) of chelate-soluble pectin (CSP) chains with significant pectinase treatments

W (nm)	Pectinase/CSP (1:1,000)		Pectinase/CSP (1:100)	
	Fq (N(%))	V (nm)	Fq (N(%))	V (nm)
11.72	–	–	7 (12.7)	0.21 ± 0.06
15.63	4 (7.1)	0.82 ± 0.22	4 (7.3)	0.90 ± 0.26
19.53	5 (8.9)	0.88 ± 0.34	6 (10.9)	0.78 ± 0.32
23.44	10 (17.9)	0.91 ± 0.28	9 (16.4)	1.08 ± 0.28
27.34	3 (5.4)	0.93 ± 0.27	3 (5.5)	1.11 ± 0.11
31.25	7 (12.5)	1.03 ± 0.42	7 (12.7)	1.10 ± 0.31
35.16	–	–	3 (5.5)	1.07 ± 0.06
39.06	3 (5.4)	1.15 ± 0.39	6 (10.9)	1.03 ± 0.30
42.97	3 (5.4)	1.14 ± 0.17	–	–
46.88	6 (10.7)	0.91 ± 0.12	1 (1.8)	0.98 ± 0.00
58.59	7 (12.5)	1.28 ± 0.22	4 (7.3)	1.26 ± 0.14
65.59	–	–	–	–
70.31	6 (10.7)	1.37 ± 0.05	5 (9.1)	1.41 ± 0.10
78.13	1 (1.8)	2.33 ± 0.00	–	–
93.75	1 (1.8)	1.98 ± 0.00	–	–
97.66	–	–	–	–
117.19	–	–	–	–
156.25	–	–	–	–
175.78	–	–	–	–
234.38	–	–	–	–

Symbol '–' means no detection or below the detection level

W, the peak width of chain half-height; V, the height of pectin chains; Fq, the number of times particular pectin chain widths were observed

Pectinase also affected the length of CSP. Figure 2 shows the frequencies of length of CSP chains with or without pectinase treatment. The length in the figure represented the length of single linear fractions (main chain) of CSP [16]. Only those linear molecules not overlapping or entangling, and fully within the scanned zone of AFM were used for statistics. The distribution of CSP lengths was in the range of 400–3,600 nm for non-pectinase-treated group, which was longer than CSP of tomato, alkali-treated pectin from sugar beet and sodium carbonate-soluble pectin (SSP) of peaches [14, 22]. This discrepancy could be due to fruit differences or partial degradation of alkali-treated pectin or SSP by β-elimination reaction [14].

During ripening, pectin modifications cause fruit softening and firmness decreasing due to pectin solubilization and hydrolysis of fruit cell wall. At least two enzymes are involved in this process: polygalacturonase (PG) and pectin methylesterase (PME) [20]. Pectin methylesterase (PME) activity of apricot fruits changes in response to different postharvest treatments [2]. PG contributes to pectin hydrolysis and possibly is responsible for pectin solubilization, while PME is responsible for the binding of cations in fruit cell wall [20]. The current work suggests that pectinase had similar effects as storage time on CSP molecules, which could be due to the effects of mixed pectin enzymes.

The length distributions of the CSP chains with different degrees of pectinase treatments are shown in Fig. 3. The results demonstrated that the largest range of apricot CSP lengths at harvest without pectinase treatment was 0.5–1.5 μm, while it was 0–1 μm for pectinase-treated groups. And in general, higher-concentration-pectinase-treated CSP chains had higher Fq of small lengths (Fig. 3). Decreased long- and increased short-CSP chains by pectinase demonstrated that pectinase facilitated the degradation of CSP in width and length. These results suggest that changes in CSP fractions associated with firmness changes in postharvest apricots might be due to pectinase effects on CSP length and width.

Figure 4 shows a schematic image of quantitative width information of CSP under pectinase treatment based on Tables 1 and 2. It revealed a quantitative relationship of widths among CSP chains. The results were similar to other fruits that the chain widths were composed of several basic units [11, 16, 17, 25, 26].

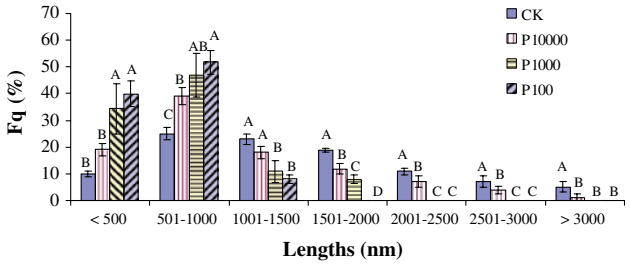

Fig. 3 Length distribution of chelate-soluble pectin (CSP) with and without pectinase treatments. Different capital letters (*A*, *B*, *C* and *D*) within same group of length indicate significant ($p < 0.05$) differences among different treatment groups with Duncan's multiple range tests

Conclusion

In vitro effect of pectinase on CSP fraction was studied. CSP chain shortened in width and length after pectinase treatment. Relative frequency of widths less than 31.25 nm was 3.6 % for control group, while it was 16.2, 52.0 and 65.5 %, respectively, for 1:10,000, 1:1,000 and 1:100 (pectinase/CSP) pectinase-treated groups. Most of the CSP lengths were 0.5–1.5 μm, while it was 0–1 μm for pectinase-treated groups. Pectinase treatment shared some similar effects on CSP fraction as storage time.

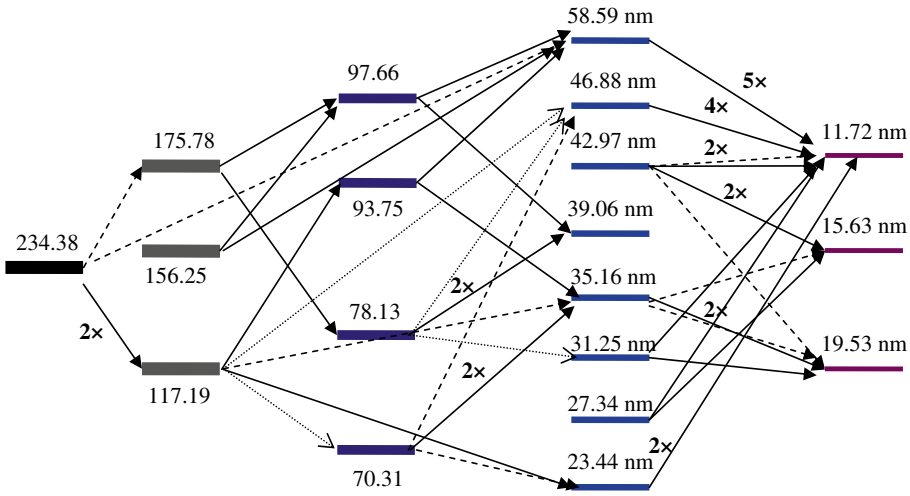

Fig. 4 Schematic image of the degradation of chelate-soluble pectin (CSP) chains with pectinase treatment in width. The numbers indicate the width of pectin chains (nm)

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Conflict of interest None.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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