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Changes in Biochemical Characteristics Related to Firmness during Fruit Development of Pepper (*Capsicum annuum* L.)

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Summary

Five pepper (*Capsicum annuum* L.) lines with varying degrees in flesh firmness were used to identify biochemical characteristics related to fruit softening. Firmness of fruit flesh (with epidermis attached) and flesh (without epidermis) was measured at different developmental stages: premature [15–20 days post-anthesis (DPA), stage 1], green mature (commercially ripe, 30–35 DPA, stage 2), colour turning (fruit becoming 30–40 % red, 40–50 DPA, stage 3), and red ripe (fruit totally red, 60–70 DPA, stage 4). Since firmness with and without the epidermis attached changed similarly in all pepper lines during development, flesh firmness (without the epidermis) was used to represent pepper fruit firmness. Biochemical characteristics measured included insoluble pectin, soluble pectin, and cellulose contents, and the activities of pectin methyl-esterase (PME), polygalacturonase (PG), β-galactosidase, and cellulase. In all lines, flesh firmness was highest at stage 3, and then decreased during development. Soluble pectin content also increased in all lines. Cellulose content normally decreased after stage 3, but these changes varied among lines. With ripening PG and PME decreased in the most firm lines, and cellulase and β-galactosidase were the key enzymes involved in the less firm lines. These biochemical characters related to fruit firmness and firmness type should provide for breeding for improvements in storage and transportation.

Key words. cellulose – cell wall hydrolases – flesh firmness – pepper lines – insoluble pectin – soluble pectin.

Introduction

A rapid decrease of flesh firmness during fruit ripening has been observed in many species including *Mangifera indica* L. (Abu-Goukh and Abu-Sarra 1993; Abu-Goukh et al. 1995), *Psidium guajava* L. (Bashir and Abu-Goukh 2003), *Pyrus communis* L. (Luton and Holland 1986), and *Malus domestica* L., *Prunus persica* L., and *Prunus armeniaca* L. (Salunkhe and Wu 1973). Fruit softening is primarily due to changes in cell-wall carbohydrate metabolism that result in a net decrease of certain structural components of cell wall in most fruits (Bartley and Knee 1982). These changes are mostly caused by hydrolytic enzymes produced in the fruit (Brummell and Harpster 2001).

Polygalacturonase (PG), pectin methyl esterase (PME), beta-galactosidase and cellulase are the major enzymes related to fruit softening (Huber 1983; White 2002). Some reports have shown that high PG activity and soluble pectin content increase concomitantly with fruit softening (Pressey et al. 1971; Awad and Young 1979; Paull and Chen 1983; Pressey 1986; Yang et al. 1993), but there is still some debate (Hadfield and Bennett 1998). Brummell and Harpster (2001) reported that solubilisation and depolymerisation of pectin appear to be two independent events. PG may play an important role in pectin solubilisation, but not necessarily pectin depolymerisation related to fruit softening. On the contrary, Xue et al. (1999) reported that increases in PG and PME activities increased soluble pectin content and resulted in rapid softening of *Prunus domestica* L fruits. The texture loss (viscosity decrease) in tomato during ripening has also been correlated with increase in PG activity (Sawamura et al. 1978). Recently, it was reported that persimmon (*Diospyros kaki*) fruit ripening took place by means of two steps in enzymatic digestion, and the key enzymes involved were PG and β-galactosidase (Nakamura et al. 2003). A coincident rapid increase of β-galactosidase activity and a rapid decrease in firmness during the final ripening stages in *Carica papaya* L. similarly indicates that β-galactosidase may play a role in fruit firmness (Manrique and Lajolo 2004).

In pepper (*Capsicum annuum* L.), excessive softening is a major post harvest problem. It causes pepper fruit shrinkage, drying and pathological disorders, and results in severe reduction in product quality and acceptability (Priya Sethu et al. 1996). It was reported that β-galactosidase plays a significant role in bell pepper fruit ripening (Ogasawara et al. 2007).

The work described here was initiated to investigate the potential relationship between biochemical characteristics and fruit firmness in various pepper lines that

differ in fruit softening. Our efforts focused on determining the contents of fruit cell wall substrates (insoluble pectin, soluble pectin and cellulose) and activities of related hydrolases (PG, PME, β-galactosidase and cellulase). This may provide some insights into the softening process, and it may also be beneficial for identifying biochemical characteristics that can be used for screening lines in pepper breeding. Early screening of characteristics related to the fruit firmness should accelerate the selection and improvement processes.

Materials and Methods

Plant material

Five pepper lines, 585 and 607 with higher firmness, and 529, 405, and 409 with lower firmness, were used in this study. Lines 585 and 529 are 'sheep horn type' hot pepper, lines 405 and 409 are 'lantern type' sweet pepper, and 607 lines is a 'small hot' pepper. The fruit firmness of a number of lines was determined one year prior to the experiment so as to choose different firmness groups. Plants were grown in soil in the greenhouse of the China Agricultural University (Beijing, China) under a day/night temperature regime of 30/20 °. Plants were watered as needed and once a week with a nutrient solution equivalent to the following rates over the course of the growing season: NH_4NO_3 100–150 kg, KH_2PO_4 5–7 kg, $ZnSO_4$ and $MnSO_4$ 1–2 kg per hectare. Fruit maturity was based on the number of days post anthesis (DPA), as well as surface coloration. Twenty plants of each line with uniform growth were used in this experiment, and the fruit from three to five plants was harvested and considered a plot. The experiment was a completely random designed. To measure firmness, cell wall composition, and hydrolase activities, fruits were harvested at four developmental stages: (15–20 DPA, stage 1), green mature (commercially ripe, 30–35 DPA, stage 2), colour turning (fruit becoming 30–40 % red, 40–50 DPA, stage 3), and red ripe (fruit totally red, 60–70 DPA, stage 4).

Firmness measurement

Fruit firmness was measured at three locations along the equatorial diameter of three representative fruits of each line and at all developmental stages. Flesh firmness was sampled similarly, but with the skin across the equatorial region of each fruit removed (at least 3 cm^2. and apart from the locations where fruit firmness were measured). A FT327 pressure tester fitted with 6.9 mm tip (Breuzzi Company, Italy) was used, and the firmness was monitored with the fruit held in hand with the tip inserted 2–3 mm into the pericarp on the exocarp side, recording the force required in grams. Three fruits were used for firmness determination, and at least three readings were taken for each fruit. The average of three readings was calculated and converted to Pa units.

Biochemical characteristics determination

Three to four fruits with the pedicel and seeds removed were cut into pieces and combined from which three replicates (sub-samples) were taken. From these replicates, about 10 g pericarp tissues were taken for enzyme determination and 20–25 g for insoluble and soluble pectin determinations. These were packed in foil and frozen in liquid nitrogen and stored at −80 °C until analysis. About 2 g from the replicates were oven-dried at 105 °C for 12 h and stored for cellulose determinations.

Insoluble pectin and soluble pectin content were analysed according to Mao and Ying (1999). Briefly, fruits were washed with distilled H_2O, excised, and sliced into small sections. 25 g minced outer pericarp tissue was first homogenized in 95 % ethanol for 3 min using a high speed masher (Electrolux LTD. Ningbo, China.), then filtered and the ethanol discarded. The filtrate was washed twice with 75 % ethanol, and then transferred to a flask, 15 ml EDTA-Na_2 added, and the pH adjusted to 11.5 with 0.1 M NaOH. After 30 min, the solution was adjusted to pH 5.5 with 2 N acetic acid, and then 15 ml water was added. The solution was incubated at 50 °C for 30 min, and after filtration, the filtrate was transferred to a 50 ml volumetric flask and made up to volume. This solution served as the soluble pectin extract. The residue after filtration was returned to a 100 ml Erlenmeyer flask, about 80 ml 0.5 M H_2SO_4 was added and incubated in boiling water for 1 h. After cooling, the solution was transferred to 100 ml volumetric flask, agitated, and made up to volume. This solution served as the insoluble pectin extract. To analyse, 1 ml extract and 6 ml H_2SO_4 was incubated in boiling water for 20 min. After cooling to room temperature, 0.2 ml 0.15 % carbazole was added, mixed well, and incubated at room temperature for 2 h. Absorbance was measured at 520 nm using a 721 UV-Visible Spectrometer (Shanghai Precision & Scientific Instrument Co. Ltd., China).

Cellulose content was assayed according to Li (2000) using about 0.2 g dried pericarp tissue.

Enzyme extraction and assay of PG (EC 3.2.1.15), PME (EC 3.1.1.11), β-galactosidase (EC 3.2.1.23), and cellulose (EC 3.2.1.4) were conducted as described by Priya Sethu et al. (1996). Acetone powder was prepared from the samples at different developmental stages of each line. Enzymes were extracted with the buffer used for the respective assay in the presence of 0.25 M NaCl (e.g., for PG, 0.15 M NaCl) at 4 °C for 12 h. The resulting suspension was passed through four layers of cheesecloth and the filtrate was centrifuged (3k30, SIGMA Centrifuge LTD. Germany) at 5000 g for 30 min. The clarified filtrate served as the crude enzyme extract.

PG activity was determined using a reaction mixture consisting of 0.2 ml enzyme extract in 0.15 M NaCl, 0.2 ml 0.2 M Tris-acetate buffer (pH 4.5), 0.1 ml 0.01 M $CaCl_2$ and 0.5 ml 1 % polygalacturonate (PGA, #457620, Sigma-Aldrich Co. Ltd., UK). A blank was prepared for each sample by boiling the reaction mixtures before addition of substrate. After 1 h at 37 °C, the reaction was stopped by heating at 100 °C for 3 min, and by adding 1 ml 1 % dinitrosalicylate reagent (DNS, Beijing Chemical Reagent Company, China). Each solution, 0.5 ml, was analysed for reducing group, the absorbance was measured at 520 nm with one unit of activity defined as the formation of 1 μM of reducing group per hour.

PME was assayed based on the procedure of Hagerman and Austin (1986). Changes in the absorbance (ΔOD) in 1 min were monitored at 616 nm. The reaction mixture contain 50 μl enzyme extract, 2 ml pH 7.5 0.5 % pectin (P7536, Sigma-Aldrich Co. Ltd., UK) solution, 0.15 ml pH

7.5 0.01 % bromothymol blue (sodium salt, Beijing Chemical Reagent Company, China) and pH 7.5 distilled water (the total solution adjusted to pH 7.5). We measured the difference in absorbance between the initial and final OD values after 60 s, and enzyme activity was expressed as ΔOD per min.

Cellulase activity was determined by measuring the reducing groups released from carboxymethyl cellulose (CMC, #C4359, Sigma-Aldrich Co. Ltd, UK) at 540 nm. The reaction mixture contained 0.25 ml of crude enzyme, 0.5 ml 100 mM NaAc (pH 5.0). Incubation was carried out at 37 °C for 2 h, and the reaction was terminated by adding 1 ml 1 % dinitrosalicylate reagent and boiling for 5 min. One unit is defined as the amount of the enzyme that catalyzed the formation of 1 μM reducing group per hour.

β-galactosidase activity was determined by measuring the liberated *p*-nitrophenyl at 420 nm after the addition of 0.5 M Na_2CO_3 to the reaction mixture with *p*-nitrophenyl β-D-galactopyranoside (#860344, Sigma-Aldrich Co. Ltd, UK) as the substrate. The enzyme reaction was performed at pH 6.6 in 0.1 M phosphate buffer solution at 37 °C for 15 min in a total volume of 3.0 ml . One unit of activity was defined as the release of one μM of substrate per hour.

Statistics

Data were analysed with One-Way ANOVA using SPSS 13.0 for Windows and means were compared with Duncan's Multiple Range Test at $P < 0.05$. Figures were drawn using Sigma Plot 10.0, and bars in the figures show standard deviation (SD) of the means with 3 replications. Correlations among all the traits were also determined using SPSS 13.0.

Results

Changes of fruit firmness and flesh firmness

Fruit firmness and flesh firmness similarly changed in all pepper lines during development. They increased till stage 3 and then began to decrease (Fig. 1). However, the extent of decrease was different, lines 585,607 and 529 decreased slowly, while lines 405 and 409 decreased markedly after stage 3. Correlations between fruit firmness and flesh firmness were significant at each stage (r=0.9944**, 0.9603**, 0.9908**, and 0.9976**, respectively.). The total correlation between fruit firmness and flesh firmness was 0.977** (Table 1); Therefore flesh firmness was used to represent the pepper firmness in this paper.

Insoluble pectin and soluble pectin content

Changes of insoluble pectin content were different among tested pepper lines (Table 2). Insoluble pectin content of line 409 reached maximum at stages 3, and

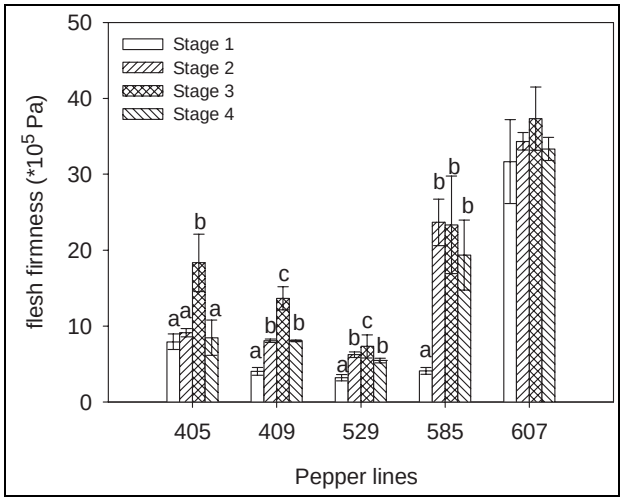


Fig. 1. Changes of flesh firmness in pepper lines 405, 409, 529, 585, and 607 during four fruit developmental stages. The letters showed the significant differences of four stages at P<0.05 in each line. Bars indicated stand deviation (SD) of three separate analyses.

Table 1. Correlation efficiency among fruit firmness, flesh firmness, insoluble pectin, soluble pectin, cellulose, PG, PME, cellulase (cellul.) activity, and β-galactosidase (β-gal.) activity of five pepper lines. Correlations among all the traits were determined using SPSS 13.0.

	Fruit firmness	Flesh firmness	Insoluble pectin	Soluble pectin	Cellulose	PG activity	PME activity	Cellul. activity	β-gal. activity
Fruit firmness	1	0.977**	0.456*	0.290	0.262	0.565*	−0.017	0.393	−0.006
Flesh firmness	0.977**	1	0.496*	0.240	0.263	0.554*	−0.144	0.371	0.010
Insoluble pectin	0.456*	0.496*	1	0.249	0.030	0.148	−0.280	0.336	0.109
Soluble pectin	0.290	0.240	0.249	1	−0.328	0.555*	0.052	0.045	0.230
Cellulose	0.262	0.263	0.030	−0.328	1	−0.156	−0.283	0.158	−0.336
PG activity	0.565*	0.554*	0.148	0.555*	−0.156	1	−0.219	−0.105	0.596**
PME activity	−0.017	−0.144	−0.280	0.052	−0.283	−0.219	1	0.336	−0.280
Cellul. activity	0.393	0.371	0.336	0.045	0.158	−0.105	0.336	1	−0.324
β-gal. activity	−0.006	0.010	0.109	0.230	−0.336	0.596**	−0.280	−0.324	1

Note: * and ** indicate significant correlation at p<0.05 and p<0.01 respectively.

Table 2. Soluble pectin, primary pectin, and cellulose content (mg g^{-1} DW) of five pepper lines in different developmental stages. Each data represents the mean ±SD (standard deviation) of three replicates.

Pectin and cellulose content	Lines	Premature stage 1	Green mature stage 2	Colour turning stage 3	Red ripe stage 4
Insoluble pectin content	405	4.92±0.73 b (B)	3.99±0.29 b (B)	3.29±0.81 d (A)	2.92±0.42 c (A)
	409	3.89±0.31 c (B)	2.49±0.58 c (A)	7.49±0.87 b (C)	2.37±0.73 c (A)
	529	2.89±0.64 d (A)	4.03±0.71 b (B)	6.41±0.76 c (C)	7.57±0.66 b (D)
	585	2.09±0.52e (B)	1.91±0.54 d (A)	1.79±0.99e (A)	2.59±0.53 c (C)
	607	8.93±0.65 a (C)	6.55±0.88 a (A)	8.73±0.53 a (BC)	8.30±0.43 a (B)
Soluble pectin content	405	3.82±0.86 c (A)	3.66±0.66 c (A)	20.47±1.23 a (C)	16.79±0.96 a (B)
	409	4.74±1.01 bc (A)	4.46±1.06 c (A)	15.31±1.12 b (C)	11.93±1.34 c (B)
	529	4.33±0.98 bc (A)	5.43±1.32 b (A)	20.18±1.38 a (C)	17.61±1.25 a (B)
	585	5.21±0.97 b (A)	10.57±0.82 a (B)	14.01±0.97 bc (C)	15.01±0.69 a (D)
	607	8.64±0.87 a (A)	10.78±0.84 a (B)	13.75±1.53 c (C)	12.91±0.75 b (C)
Cellulose content	405	1.71±0.32 d (C)	2.01±0.47 c (D)	1.61±0.36 d (B)	1.41±0.45 d (A)
	409	2.31±0.27 b (C)	2.19±0.33 b (B)	2.71±0.45 a (D)	1.99±0.28 a (A)
	529	2.37±0.32 a (D)	0.98±0.41 e (C)	0.76±0.49 e (B)	0.69±0.35 e (A)
	585	1.27±0.19 e (A)	1.58±0.12 d (B)	1.92±0.21 c (C)	1.65±0.38 b (B)
	607	1.96±0.45 c (B)	2.56±0.56 a (D)	2.18±0.34 b (C)	1.54±0.29 c (A)

Note: Means followed by different small letters are significantly different at P<0.05 between the lines at the same developmental stage, means followed by different upper case letters in () indicated the significant differences at p<0.05 between the stages at the same line

then decreased. In line 529, insoluble pectin content increased throughout fruit development, while there were no such changes among lines 607, 585, and 405. Insoluble pectin contents were significantly different between lines 607 and 585 at every stage (Table 2). Line 607 had the highest insoluble pectin content while line 585 had the lowest among all tested lines. The correlation between fruit firmness and insoluble pectin was significant (r=0.496*, Table 1). As firmness decreased, insoluble pectin content significantly decreased in line 409; but significantly increased in line 529, and changed only slowly in lines 607 (Table 2). This suggested that the insoluble pectin content presumably contributes to pepper fruit firmness, but the influence varied among lines.

Fruit soluble pectin content tended to increase during the first three stages, and then decreased, except line 585 reached a maximum at the stage 4 (Table 2). Soluble pectin content in lines 607 and 585 changed slightly during fruit development, while that in lines 405, 409, and 529 dramatically increased from stage 2 to stage 3, suggesting that the soluble pectin content might be relate to the fruit firmness decrease. However, the correlation between flesh firmness and soluble pectin was not significant (r=0.240, Table 1).

Changes of PG, PME, and β-galactosidase activities

Maximum PME activity occurred earlier than PG activity. PME activity peaked at stage 2 in line 585 and at stage 3 in other four lines, but then decreased (Fig. 2A). PG activities in fruits of all pepper lines investigated increased throughout fruit development (Fig. 2B).

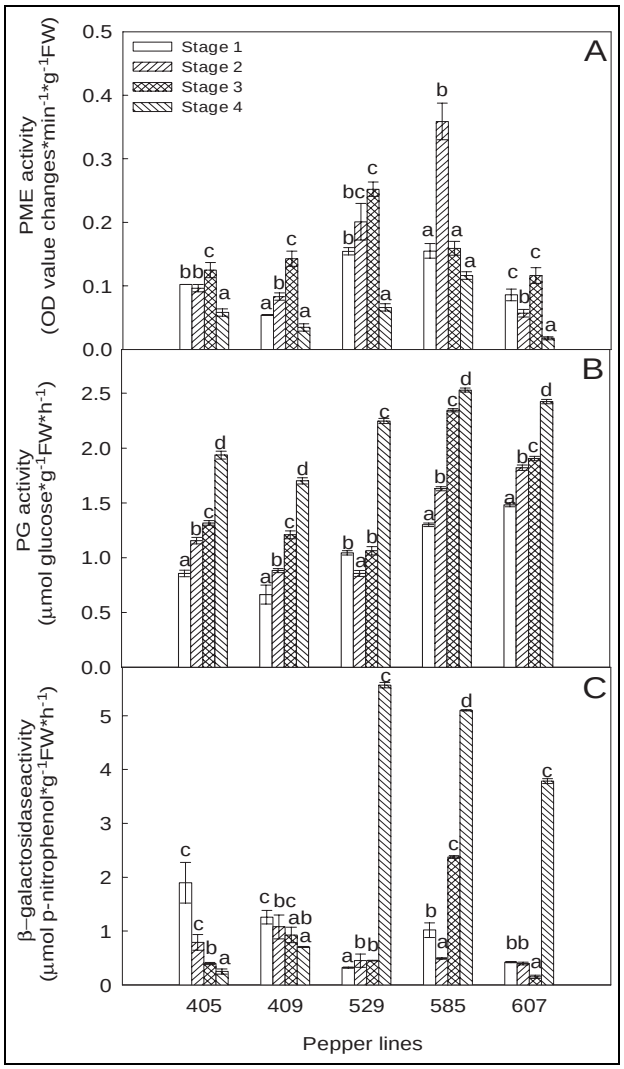


Fig. 2. Changes of polygalacturonase (PG) activity (A), pectinmethyl-esterase (PME) activity (B), and β-galactosidase activity (C) in fruits of pepper lines 405, 409, 529, 585, and 607 during four fruit developmental stages. The letters showed the significant differences of four stages in each line at P<0.05. Bars indicated stand deviation (SD) of three separate analyses.

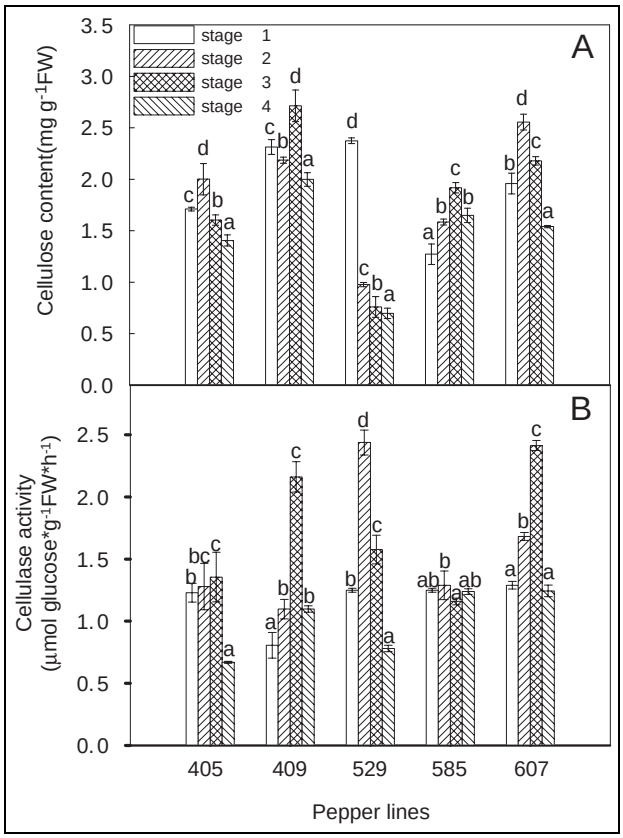


Fig. 3. Changes of cell wall cellulose content (A) and cellulase activity (B) in fruits of pepper lines 405, 409, 529, 585, and 607 during four fruit developmental stages. The letters showed the significant differences of four stages in each line at P<0.05. Bars indicated stand deviation (SD) of three separate analyses.

β-galactosidase activity increased significantly from stage 2 or 3 to stage 4 in three hot pepper lines (529, 585, and 607), while it slightly decreased in lines 405 and 409 (Fig. 2C).

Changes of cellulase activity and cell wall cellulose content

There was no significant correlation between total cellulose content and cellulase activity (r=0.158, Table 1), and between total cellulose and firmness (r=0.263, Table 1). Cellulose content of line 529 dramatically decreased at the beginning of fruit development in parallel with the firmness decrease. In lines 585 and 607, firmness decreased concomitantly with the cellulose content from stage 2 or stage 3. However, in line 409 and 405, the cellulose content change was otherwise (Fig. 1, Fig. 3A, and Table 1). This indicated that changes of cell wall cellulose content might affect the fruit firmness in lines 529, 585, and 607, but had less influence in lines 405 and 409.

The cellulase activity reached maximum at stage 3 in lines 607, 405, and 409, at stage 2 in lines 529 and 585, and then declined (Fig. 3B). The cellulose content decreased when cellulase activity increase in line 529 (stage 2), and 607 (stage 3), in parallel with the firmness decreases. The decrease in cellulose content of line 585 also accompanied the firmness decrease, but cellulase activity changed only slightly. There were no obvious correlations in line 405 and 409 between cellulase activity and cellulose content. The firmness decrease at stage 4 may instead have been due to β-galactosidase activity on the fruit pectin content.

Fruit firmness changes in 607, 585, and 529 lines (hot chili types) were closely correlated with cellulose content and cellulase activity, but cellulose content and cellulase activity changes had less apparent influence on firmness in 405 and 409 lines ('lantern type' sweet pepper lines with lower firmness).

Discussion

PG activity has long benn generally considered to be closely related to the fruit softening although its content

varies in fruits of different species (Pressey et al. 1971; Awad and Young 1979; Paull and Chen 1983; Pressey 1986; Yang et al. 1993; Xue et al. 1999). However, several studies with transgenic tomatoes, in which PG activity was suppressed, indicated that PG activity alone is not sufficient to affect fruit softening and ripening significantly, and softening is not solely a PG-dependent process. PG-mediated pectin depolymerisation was not necessary for normal ripening and softening (Sheehy et al. 1988; Smith et al. 1988; Giovannoni et al. 1989). Recently, Vicente et al. (2007) reported that several tomato mutants whose fruits exhibit exceptional shelf life, remaining firm, and at an edible texture for a long time after fully ripening, but cell wall degradation and cell-cell separation occur to the same extent and at the same rate as in normally softening fruits, suggesting that cell wall disassembly is not the sole and primary cause of fruit softening. The specific contribution of PG in tomato fruit ripening still remains an open question (Tomassen et al. 2007). Other recent results show that in tomato, PG and expansin are among the cell wall proteins that cooperatively participate in ripening associated cell wall disassembly (Cantu et al. 2008). This cooperative action of PG and expansin apparently affects the integrity of fruit cell wall polysaccharide networks and cause cell wall relaxation.

In hot pepper, compared with a number of other fruits, cell wall uronic acid content shows only a slight change after an initial decrease from the immature green to the mature green stage. The relative cellulose content decreases during this period prior to maturity, but then slightly increases (Gross et al. 1986). In bell pepper, fruit softening isn't correlated with PME activity since PME declines as softening increased (Priya Sethu et al. 1996). The mechanisms causing fruit softening during pepper development, are not that well understood, and among pepper lines there are differences in fruit firmness changes that are somewhat contradictory.

In this study, the activities of PG increased during fruit development and soluble pectin content was maximal at the stage 3 or 4 in lower firmness lines 405 and 409. Thus, increases in PG activity might be the main cause for the changes in the soluble pectin content from stage 3 to stage 4 when, concurrently, fruit firmness decreased. All this suggests that fruit firmness was closely related to the increase of soluble pectin content modified by the increase of PG activity. In addition, the soluble pectin content increase in line 529 was concomitant with the increase of insoluble pectin content, suggesting that in these cell walls degradation and synthesis occur simultaneously during pepper fruit development and ripening. The fruit firmness decrease in line 529 was not consistent with a decrease in cell wall pectin content, suggesting that in this line fruit firmness was not directly correlated with pectin content, or the activities of PG, and PME. This suggests in this line that the cell wall degradation during fruit development and ripening can't be attributed to only one or two factors. Changes of fruit firmness were significantly correlated with the increase of soluble pectin content at stages 3 and 4 in line 405 and 409 ($r=-0.6728$ and -0.4675, respectively), therefore, it is proposed that the soluble pectin content might have a higher effect on fruit firmness in 405, 409 lines than that in the 607 and 585 lines.

Related to these results, Carrington and Pressey (1996) found that the cell wall materials change varies in different lines of the same tomato species. Hiwasa et al. (2004) reported that in pear fruit, cell wall degradation is correlated with a decrease in firmness during ripening and the modification of both pectin and hemicellulose are essential for the development of a melting texture. Furthermore, it was suggested that different softening behaviours during ripening among the three pear fruits may be caused by different endo-PG activity and different expression of PG genes. Our results were consistent with their findings. Fruit firmness was highly correlated with cell wall pectin content, PG, and PME activity in lower firmness lines (the 'lantern type' sweet pepper lines 405 and 409), but not in the higher firmness lines (the 'hot pepper' lines 607, 585,) and lower firmness line 529('sheep horn type' hot pepper). This indicates, as in other species, that in these distinctly different pepper types, the cell wall pectin degradation, PG, and PME activity might have different roles in fruit firmness changes.

PME action was believed to be a prerequisite for PG action (Wang and Han 1998), and more recently, pectin methylesterase activity in vivo was shown to differ from activity in vitro and to enhance polygalacturonase-mediated pectin degradation in 'tabasco' pepper (Arancibia and Motsenbocher 2006). In this study, the PG activity in fruits of lines 607 and 585 with higher firmness were higher than in other lines (Fig. 2B), but the soluble pectin and the insoluble pectin contents changed slowly. Low PME activity in line 607 may limit its PG acting on the substrate insoluble pectin. In line 585, PME activity reached the highest level at stage 2, while the PG activity was low at this time. When the PG activity increased, the PME activity decreased and there was a slow change in insoluble pectin and soluble pectin content. On the other hand, the PME activity in the lower firmness lines 529, 405, and 409 reached a maximum before PG activity rapidly increased. Moreover, the abundant soluble pectin content due to the PG activity increase resulted in the decrease of insoluble pectin content (Table 2) and lower fruit firmness.

We have concluded that fruit firmness changes were to some extent correlated to the soluble pectin and cellulose content during development and ripening. However, the key biochemical characteristics causing fruit firmness changes were clearly different among the pepper fruit types. In 'hot pepper' lines, fruit firmness change was closely correlated with the cellulose content, activities of cellulase and β-galactosidase, while in 'lantern type' sweet pepper lines, fruit firmness changes was closely correlated with the insoluble pectin content, soluble pectin content, and activities of PG and PME. Therefore in pepper breeding, these biochemical characteristics may provide some guides in screening lines for postharvest storage and handling tolerance. It may be possible to select the lines at early fruit developmental stage according to their biochemical characteristics, decreasing the breeding cycle and breeding costs.

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