Changes in Pectic Polysaccharides during the Ripening of Cherry Tomato Fruits

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Pectic polysaccharides in cherry tomato fruits (Pepe) have been studied at three stages of ripening (immature-green, mature-green, and mature-red). The alcohol-insoluble solids obtained from the fruits were fractionated into four groups of pectic polysaccharides: water-soluble pectin (WP), hexametaphosphate-soluble pectin (PP), HCl-soluble pectin (HP), and KOH-soluble pectin (KP). The content of total pectic polysaccharides decreased with ripening of the fruits; especially, the main fraction HP at immature-green stage hardly existed at the mature-red stage. Instead, the content of WP increased with maturation. The pectic polysaccharide fractions were analyzed by gel-filtration, and the molecular weight of each fraction decreased with ripening. This phenomenon was accompanied by an increase in the activities of the pectin-hydrolyzing enzymes, polygalacturonase and pectinesterase. These results indicate that the softening of cherry tomato fruits during the ripening may depend on degradation and depolymerization of pectic polysaccharides by the pectin-hydrolyzing enzymes.

Keywords: pectin, cherry tomato fruits, ripening, polygalacturonase, pectinesterase

The softening of fruit tissues is thought to be due to the physiological changes of polysaccharide constituents including pectic polysaccharides. The pectic polysaccharides are hetero-polysaccharides and are the major components of the middle lamella and the primary cell walls of fruit tissues (Tuji & Mori, 1997). The pectin in immature fruits is water-insoluble protopectin, which decomposes into water-soluble pectin during maturation. Therefore, pectic-hydrolyzing enzymes such as pectinesterase and polygalacturonase are assumed to contribute primarily to the softening of fruit tissues through the decomposition of pectic polysaccharides.

Tomato is one of the most popular fruits and vegetables in the world. The pectin-hydrolyzing enzymes of tomato fruits have been investigated in connection with fruit softening (Watson *et al.*, 1994; Gross & Wallner, 1979; Grierson & Tucker, 1983; Brady *et al.*, 1982). In our previous study, we reported the purification and some properties of pectinesterase from tomato fruits of a miniature-fruited red type (cherry tomato) (Inari *et al.*, 2000). The activities of this enzyme in cherry tomato fruits increased during ripening, and were higher than other fruits, such as strawberry, banana, persimmon, tomato (cv Momotaro). We were therefore interested in cherry tomato, and such a pectin-hydrolyzing enzyme has been thought to be an important factor affecting fruit softening. In this report, we studied the compositional changes of pectic polysaccharides during the maturation of cherry tomato fruits.

Materials and Methods

Tomato fruits Cherry tomato plants (cv Pepe) were grown in the field. The fruits were harvested at three stages of maturation: immature green (IG, green and smaller fruits, less than 8 g/fruit), mature green (MG, green and some white and larger fruits,

more than 8 g/fruit), and mature red (MR, red and suitable for eating, more than 8 g/fruit) stages depending on the level of pigment development. The mean weight of the fruit of these respective stages was 3.6 g, 9.7 g and 12.8 g. Although the degree of fruit ripening is generally judged from the number of days after flowering or the degree of coloration, we used peel color to determine ripening (Abe *et al.*, 2000). The uniformity of the degree of ripening among the fruits was beneficial in our conduct of the study.

Preparation of pectic polysaccharides Alcohol insoluble solids (AIS) were prepared from each fresh tomato fruit harvested at one of the three stages of maturation. The AIS was sequentially extracted with water (water-soluble pectin, WP), 0.4% hexametaphosphate solution (hexametaphosphate-soluble pectin, PP), 50 mM HCl solution (HCl-soluble pectin, HP), and 50 mM KOH solution (KOH-soluble pectin, KP) (Inari & Takeuchi, 1997).

Sugar analysis Total sugar contents were determined by the phenol-sulfate method, and galacturonic acid content by the carbazol-sulfate method (Fukui, 1979). Methoxyl contents were determined as previously reported (Inari & Takeuchi, 1997). The compositions of neutral sugars were analyzed by HPLC method after hydrolysis with 1 M H₂SO₄ (Inari & Takeuchi, 1995).

Mineral analysis Content of the minerals Cu, Ca, Fe, K, Mg, and Na in AIS was measured by an atomic absorption spectrophotometer (Shimadzu AA-6500F) after hydrodecomposition.

Gel filtration The molecular weight of each pectin fraction was estimated by gel filtration using a Cellulofine GCL-2000-m column (3.0×80 cm) with 0.1 M NaCl solution at a flow rate of 1 ml/min. Dextran (Mw. 2,000,000, 40,800, and 11,000), amylose (Mw. 294,000 and 118,000), and lactic acid (Mw. 360) were used as the standards.

Polygalacturonase activity The fruits were homogenized in cold distilled water containing 2% polyvinylpyrrolidone. The

homogenate was centrifuged at 20,000×*g* for 10 min at 4°C, and the precipitate pellet was then suspended in 1 M NaCl solution at pH 6.0. After stirring at 4°C for 3 h, the suspension was centrifuged at 20,000×*g* for 10 min, and the supernatant fluid was used as the polygalacturonase sample. Commercial polygalacturonic acid (Sigma Chemical Co.) was dissolved in 50 mM sodium acetate buffer (pH 3.8) at a concentration of 0.2% and used as the substrate. The enzyme solution was added in 1.0 ml of the substrate solution and incubated at 40°C. The resulting reducing sugars were measured by the method of Somogy-Nelson (Tucker *et al.*, 1980). One unit of the polygalacturonase activity was defined as the corresponding amount of 1 µmol/min of galacturonic acid released.

Pectinesterase activity The fruits were homogenized in 10% NaCl solution containing 2% polyvinylpyrrolidone. The homogenate was adjusted at pH 7.5, allowed to stand for 1 h at 50°C to autolysis, and centrifuged at 20,000×*g* for 20 min in a refrigerated centrifuge. The supernatant fluid was dialyzed against 1% NaCl solution overnight and was designated as crude extract; this extract was fractionated by ammonium sulfate precipitation at 40% saturation at 4°C. The resulting precipitate collected by centrifugation was then dissolved in 10% NaCl solution and dialyzed against 10 mM sodium phosphate buffer at pH 6.0. Commercial citrus pectin was dissolved in 100 mM NaCl solution at a concentration of 1% and used as the substrate. The measurement of pectinesterase activity was based on the amount of consumed 0.1 M NaOH solution to neutralize the pectic acid

produced from pectin at 50°C for 10 min. One unit of the pectinesterase activity was defined as the corresponding amount of 1 µM methoxyl group for 10 min (Inari *et al.*, 2000).

Hardness of cherry tomato fruits Hardness of the fruit tissues was measured by a creep meter (Yamaden RE-330). The fruit samples were stood on their did calyx upside down and cut to a center thickness of 1 cm. The sarcocarp was measured to determine hardness of the cherry tomato fruit. Conditions of measurements were a cylinder style plunger with a diameter of 5 mm, compressibility of 20%, voltage of 1.0–5.0, and speed of 5 mm/s.

Results and Discussion

In this work, we used three ripening stages of cherry tomato fruits, IG, MG, and MR stages. The hardness of each fruit tissue was measured using a creep meter (Table 1), and fruits of the MR stage showed the lowest hardness values among the tissues.

The AIS content of cherry tomato fruits at IG, MG, and MR

Table 1. Changes in fruit hardness during the ripening of cherry tomato fruits.

Stage	Hardness (×10 ⁵ N/m ²)
Immature green	6.06±0.26 ^{a)}
Mature green	5.20±1.15
Mature red	1.38±0.19

^{a)}Mean±SD (*n*=5).

Table 2. Mineral contents of alcohol insoluble solids (AIS) from cherry tomato fruits.

Stage	Mineral (mg/g AIS)						
	Mg	Ca	Na	K	Cu	Fe	Total
Immature green	0.61±0.11	5.78±1.43	0.24±0.02	6.39±0.71	tr.	0.16±0.01	13.18
Mature green	1.85±0.24	2.76±0.92	0.16±0.02	5.67±0.77	tr.	0.21±0.01	10.65
Mature red	2.12±0.12	2.71±1.05	0.29±0.02	8.04±0.15	tr.	0.24±0.02	13.40

tr., trace amount (less than 0.004 mg/g AIS wt.).

The values are expressed as mean±SE of 3 replicates.

Table 3. Sugar contents of pectic polysaccharide fractions from alcohol insoluble solids (AIS) of cherry tomato fruits.

Stage	AIS fraction	Uronic acid ^{a)} (mg/g fw)	Neutral sugar ^{b)} (mg/g fw)							Total pectin content ^{c)}		Methoxyl content (%)
			Ara	Glc	Xyl	Gal	Man	Rha	Sub-total	(mg/100 g fw)	(%)	
Immature green	WP	16.9±0.2 ^{d)}	1.5	8.7	1.4	4.9	1.4	0.1	18.0	34.9	(5.1)	12.0±0.6 ^{e)}
	PP	59.9±2.4	1.3	0.8	0.8	2.2	0.1	0.7	6.0	66.0	(9.7)	6.7±0.7
	HP	258.6±10.2	19.6	166.5	2.1	96.3	2.4	6.7	293.6	552.2	(81.1)	16.3±0.9
	KP	15.5±1.2	0.7	4.8	2.9	2.3	0.4	0.7	11.9	27.4	(4.0)	— ^{f)}
	Total	351.0	23.2	180.9	7.2	105.7	4.3	8.3	329.5	680.5	(100.0)	14.4
Mature green	WP	55.0±2.3	1.5	1.3	1.5	3.1	1.4	0.4	9.3	64.3	(17.7)	9.7±0.6
	PP	101.7±9.0	3.7	0.0	0.2	12.0	0.4	2.2	18.5	120.2	(33.0)	6.3±0.4
	HP	97.3±1.9	3.5	32.9	n.d. ^{g)}	10.9	3.8	1.2	52.2	149.5	(41.1)	16.3±0.8
	KP	22.7±0.9	1.2	0.8	2.9	1.3	0.4	0.4	7.1	29.8	(8.2)	—
	Total	276.7	9.9	34.9	4.6	27.3	6.1	4.2	87.0	363.7	(100.0)	10.8
Mature red	WP	102.0±5.0	1.8	1.0	1.4	4.7	0.8	2.6	12.2	114.1	(66.1)	9.2±0.2
	PP	11.6±0.3	0.6	n.d.	n.d.	0.4	0.0	0.5	1.5	13.0	(7.5)	6.8±1.9
	HP	11.8±0.2	3.1	2.0	n.d.	3.6	5.0	0.7	14.4	26.1	(15.1)	13.4±1.0
	KP	13.0±0.4	1.3	0.4	1.4	2.0	0.6	0.6	6.3	19.3	(11.2)	—
	Total	138.2	6.7	3.4	2.7	10.7	6.3	4.5	34.3	172.6	(100.0)	9.4

^{a)}Analyzed by the carbazol-H₂SO₄ method.

^{b)}Analyzed by the HPLC method as described in Materials and Methods.

^{c)}Total values of uronic acid plus sub-total of neutral sugars.

^{d)}Mean±SD (*n*=3).

^{e)}Mean±SD (*n*=3).

^{f)}Since KP was partially saponified during the extraction, the methoxyl content could not be determined.

^{g)}n.d., not detected.

stages was 2.32, 2.12, and 1.76 g per 100 g of fresh weight, respectively. It is believed AIS content is large because AIS in IG is involved with starch, cellulose, hemicellulose, lignin and protein. The AIS content in MR is believed to decrease because the alcohol soluble solids such as the sugar of a low molecule and pigment increase when the fruit ripens. Since the AIS of cherry tomato decreases with ripening it may be governed by the softening of the fruit.

The mineral contents in each AIS are shown in Table 2. We measured the six mineral components, magnesium, calcium, sodium, potassium, copper, and iron, and found that the content of each hardly changed during ripening. Pectin has cross-linkage within the pectic carboxyl residue with divalent cations such as Ca or Mg. Van Buren (1979) reported that an increase in calcium and magnesium concentrations might be related to an increase in firmness of fruits and vegetables. Tieman and Handa (1994) indicated that the amounts of Ca and Mg in tomato fruits decreased during ripening and development could be due to the destruction of cross-links between galacturonic acid units of adjacent pectic chains. However, our results showed the amounts of AIS minerals did not change a really during the maturation of cherry tomato fruits. The amounts of Ca associated with AIS decreased slightly during development, while the amounts of Mg and Fe increased a little. In other words, the total amounts of divalent cation hardly changed. So the mineral components in AIS may

not contribute to the softening in cherry tomato fruits.

Each AIS was fractionated into four types of pectic polysaccharides, WP, PP, HP, and KP. Table 3 shows the sugar compositions and methoxyl contents in each type during the three stages

Fig. 2. Changes in polygalacturonase (PG) and pectinesterase (PE) activities of cherry tomato fruits during maturation. Values are expressed as mean $\pm$ SE of four replicates. The data of PE (●) were quoted from our previous work (Inari *et al.*, 2000). ▨, PG activity.

Fig. 1. Gel-permeation chromatograms of pectic polysaccharide fractions from cherry tomato fruits.

The pectin solution was loaded on a Cellulofine GCL-2000-m column (3.0 $\times$ 80 cm) eluted with 0.1 M NaCl solution at a flow rate of 1 ml/min. The eluate was collected in 10-ml fractions and analyzed for galacturonic acid by the carbazol-sulfate method at 530 nm. Arrows indicate the eluted positions of the standard samples. ▲, immature green; ●, mature green; ■, mature red.

of ripeness. The most abundant polysaccharide fraction in the IG stage was HP, which consisted of almost equivalent amounts of uronic acid and neutral sugars (mainly glucose and galactose) and was high in methoxyl content. This fraction decreased with maturation. The main fraction in the MR stage was WP, which consisted mainly of uronic acid and had lower methoxyl content. The results indicate that the HP fraction in the IM stage might change into the WP fraction with fruit maturation. Gross and Wallner (1979) reported that the components declining in amount were galactose, arabinose, and galacturonic acid of cell wall polysaccharides during the ripening of tomato fruits. Redgwell *et al.* (1997) discussed the relationship between pectin solubility and galactose loss and their respective contribution to fruit softening. In the present study, the same results were obtained from the fruits of cherry tomato. The methoxyl content was also shown to decrease with fruit ripening (Table 3). We have reported that the activity of pectinesterase in cherry tomato fruits increased with ripening (Inari *et al.*, 2000). Thus, the decrease of methoxyl content during the ripening might be due to a rise in pectinesterase activity.

Figure 1 shows the gel-permeation chromatogram of each pectin fraction. The molecular weights of WP at IM, MG, and MR stages were calculated to be 408,000, 360,000, and 4,000; those of PP to be 1,524,000, 49,000, and 38,000; those of HP to be 49,000, 28,000, and 4,000; and those of KP to be 8,000, 13,000, and 22,000, respectively. Fisher and Bennett (1991) reported that the soluble pectin underwent some depolymerization and the average molecular weight significantly decreased during ripening. Our results indicated that HP, the main pectin at IM the stage, lowered in both content and molecular weight with ripening. In addition, the molecular weight of WP, the main pectin at the MR stage, also dropped with ripening. The molecular weight of cherry tomato total pectin was calculated on the basis of the organization ratio of the total pectin content of Table 3, and was IM 315,000, MG 104,000 and MR 9,000. We then measured the activities of pectin-hydrolyzing enzymes during the fruit ripening. Figure 2 shows the polygalacturonase and pectinesterase activities of cherry tomato fruits during the three ripening stages; the activities of both the pectin-hydrolyzing enzymes increased. Brady *et al.* (1982) measured the activities of pectinesterase and polygalacturonase and the content of water-soluble uronide in tomato fruits at different stages of ripening. They indicated that pectinesterase did not vary with maturity although the activities of polygalacturonase increased and the contents of water-soluble uronide were similar to polygalacturonase. In contrast, our present study showed the activities of pectin-hydrolyzing enzymes increased with the depolymerization of pectic polysaccharides. Thus, these enzymes can contribute to the softening of cherry tomato fruits through the decomposition of pectic polysaccharides.

There are some fruits in which the pectin depolymerization

occurs during ripening. The softening of fruits during ripening is assumed to be due to a lowering of the pectin molecule or to a decrease in its content. Gross (1990) reported that changes in the neutral sugar moiety in the pectin molecule modified the solubility of pectin. Batisse *et al.* (1994) reported that the softening of cherry fruits depends on the changes of neutral sugar moiety of the pectin molecule. Therefore, the changes of neutral sugar moiety in pectin may be an important factor in addition to the depolymerization by pectin-hydrolyzing enzymes.

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