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Modification of pectin polysaccharides during ripening of postharvest banana fruit

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ABSTRACT

Pectin is one of the major components of the primary cellular walls and middle lamella in plant tissues. In this study, water-soluble pectin (WSP) and acid-soluble pectin (ASP) fractions were isolated from pulp tissues of banana fruit at various ripening stages. Their monosaccharide compositions, glycosyl linkages and molecular mass distributions were evaluated. As ripening progressed, fruit firmness decreased rapidly, which was associated with the increase in the WSP content and the decrease in the ASP content. Meanwhile, the molecular mass distributions of WSP and ASP fractions exhibited a downshift tendency, indicating the disassembly of pectin polysaccharides. Moreover, galactose and galacturonic acid as the major monosaccharide compositions of pectin polysaccharides increased in WSP fraction but decreased in ASP fraction during fruit softening. GC–MS analysis further revealed that pectin polysaccharide had a 1,4-linked galactan/galacturonan backbone with different types of branching and terminal linkages in WSP and ASP fractions. During banana fruit ripening, the amount of 1,4-linked Gal*p* residues of ASP fraction decreased significantly whereas 1,3,6-linked Gal*p*, 1,2-linked Man*p* and 4-linked Ara*f* residues disappeared, which was related to depolymerization of pectin polysaccharides. Overall, the study indicated that the modifications in polysaccharide compositions and glycosyl linkages, reduced molecular mass distributions and enhanced depolymerization of pectin fraction during banana ripening were responsible for fruit softening.

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1. Introduction

Pectin is one of the major components of the primary cellular walls and middle lamella in plant tissues. The pectic matrix provides an environment for the deposition, slippage and extension of the cellulosic-glycan network and is the major adhesive materials between cells (Willats, McCartney, Machie, & Knox, 2001). Pectin degradation plays an important role in fruit ripening, which leads to disassembly of cellulose and hemicellulose network and decrease in fruit firmness.

Involvement of polygalacturonase (PG) or/and pectin methyl esterase (PME) in enzymatic disassembly of pectin polysaccharides during fruit ripening has been extensively investigated (Nikolic & Mojovic, 2007; Prasanna, Prabha, & Tharanatha, 2007; Verlent, Smout, Duvetter, Hendrickx, & Loey, 2005). PG and PME cooperatively regulate the pectin disassembly. PG hydrolyses α-1,4-linked D-galacturonic acid, following de-esterification of pectin by PME. In addition, pectate lyase (PL) (Payasi, Misra, & Sanwal, 2006) and β-galactosidase (Lazan, Ng, Goh, & Ali, 2004) may play a combined effect on the disassembly of pectin polysaccharides during fruit ripening/softening.

Non-enzymic action might mediate polysaccharide solubilisation in plant tissues (Dumville & Fry, 2003). Schopfer (2001) and Schweikert, Liszkay, and Schopfer (2002) reported that hydroxyl radical (·OH) was capable of cleaving polysaccharides present in cellular walls in a site-specific reaction, which led to cellular wall loosening and elongation of living coleoptile or hypocotyls in maize and soybean. Ascorbate, especially in the presence of traces of either Cu^{2+} or H_2O_2, can promote markedly the non-enzymic solubilisation of pectin and xyloglucan from plant cellular walls *in vitro* (Dumville & Fry, 2003; Miller & Fry, 2001).

Enzymatic and non-enzymatic effects on pectin degradation are associated with structural modification, including the changes in the composition, molecular weight and structural characteristic of the pectin. There are some reports on modification of cellular wall polysaccharides in apple (Scalzo, Forni, Lupi, Giudetti, & Testoni, 2005), peach (Manganaris, Vasilakakis, Diamantidis, & Mignani, 2006), papaya (Manrique & Lajolo, 2004) and tomato (Reinders & Their, 1999). However, variations in cellular wall compositions could lead to differences in the softening-associated chemical modification for each fruit species.

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Further investigation into pectin degradation with emphasis on its structural modifications and characteristics during fruit ripening/softening is needed.

Banana is a typical climacteric fruit, which is characterized by rapid softening once ripening is initiated (Duan, Joyce, & Jiang, 2007; Jiang, Joyce, & Macnish, 1999; Wills, Klieber, David, & Siridhata, 1990). Although characteristic of fruit softening has been established, little information on the structural characteristics and modifications of the cellular walls during softening of banana fruit is available. The objective of this study was to investigate the changes in the polysaccharide compositions, glycosyl linkages and molecular mass distributions of banana fruit during ripening, in relation to depolymerization of pectin materials, for further elucidation of fruit softening.

2. Materials and methods

2.1. Plant materials

Hands of mature green banana fruit (*Musa* spp., AAA group, cv. Brazil) were obtained from a local farm in Guangzhou. Fruits were cut into fingers and then dipped in 0.1% Sportak® (prochloraz, Bayer) fungicide solution for 3 min to control the postharvest diseases. After air-dried for 1 h, the fruits were kept at 25 ± 1 °C and 90% relative humidity. At 0, 10, 15, 17 and 20 days when fruit ripening reached stage I (mature green), II (green), III (green > yellow), IV (yellow > green) and V (yellow), respectively, 10 fingers of these fruits were sampled and then peeled. The pulp tissues were cut into small pieces, frozen immediately in liquid nitrogen and finally stored at −80 °C.

2.2. Preparation of alcohol-insoluble residue

The alcohol-insoluble residue (AIR) was prepared according to the method of Vierhuis, Schols, and Beldman (2000) with some modifications. The frozen pulp tissues (100 g) were blended for 2 min with 300 ml of 95% (v/v) ethanol using a homogenizer, and then maintained in boiling water for 15 min to inactivate endogenous enzymes. After cooling rapidly in ice bath, the homogenate was centrifuged at 4000*g* for 15 min. The residue was washed sequentially with 200 ml of mixture solution of chloroform: methanol (1:1, v/v) and 200 ml of acetone. Banana pulp starch was removed by re-extracting overnight in 90% aqueous Me_2SO and no starch was detected using the KI-I_2 method (Nelson, 1968). The extract suspension was then centrifuged at 4000*g* for 15 min. The precipitate was washed twice with 70% ethanol at room temperature (about 25 °C), then filtered and finally dried at 40 °C. The dried powder was considered as alcohol-insoluble residue and then stored in desiccators.

2.3. Fractionation of pectin polysaccharides

AIR from banana fruit at different ripening stages was subjected to sequential fractions by the method of Majumder and Mazumdar (2002) with some modifications. About 5 g of AIR was stirred for 1 h with 30 ml of distilled water at 25 °C. The suspension was centrifuged at 4000*g* for 15 min, and the residue was then re-extracted twice. The combined supernatants were filtered through Whatman filter paper (No. 1) and lyophilized to obtain water-soluble pectin (WSP). Afterwards, the water-insoluble fraction was treated for 1 h with 100 ml of 0.5 M HCl at 60 °C and centrifuged for 15 min at 4000*g*. The supernatant was collected, filtered through fritted glass, dialyzed against distilled water, and then lyophilized to obtain acid-soluble pectin (ASP).

2.4. Galacturonic acid and neutral sugar analysis

The analysis of monosaccharide compositions was conducted by the method of Yang et al. (2006) with minor modification. The polysaccharide sample (10 mg) was hydrolyzed for 6 h with 10 ml of 2 M trifluoroacetic acid (TFA) at 120 °C. Derivation was then carried out using the trimethylsilylation reagent. The trimethylsilylated derivatives were loaded onto a HP 6890 gas chromatograph (GC) equipped with a HP-5 capillary column (30 m, 0.32 mm, 0.25 μm) and a flame-ionization detector (FID), using inositol as the internal standard. The GC was performed using the following conditions. H_2: 30 ml/min; air: 150 ml/min; N_2: 1 ml/min; injection temperature: 230 °C; detector temperature: 230 °C; column temperatures programmed from 130 to 180 °C at 5 °C/min, holding for 2 min at 180 °C, then increasing to 220 °C at 5 °C/min and finally holding for 3 min at 220 °C. The monosaccharides (arabinose, xylose, galactose, glucose, rhamnose, mannose and fructose) and uronic acid (galacturonic acid) were used as standards.

2.5. Analysis of the glycosidic linkages

The glycosidic linkage analysis was carried out by the method of Kim, Reuhs, Michon, Kaiser, and Arumughama (2006) with minor modification. Briefly, the dried sample (4.0 mg) was dissolved with 2 ml of Me_2SO under nitrogen condition and then methylated with 1.5 ml of CH_3I and 20 mg of NaOH powder. Partially methylated alditol acetates were prepared from fully methylated samples by acid hydrolysis with 2 M TFA at 120 °C for 1 h, with the reduction of the hydrolysates using $NaBH_4$ followed by acetylation with acetic anhydride. The alditol acetates were analyzed by GC–MS (QP2010 Plus), using EC™-5 capillary column (30 m, 0.25 mm, 0.25 μm). Helium was used as carrier gas at a constant flow rate of 1 ml/min. The oven conditions included an initial temperature of 80 °C for 2 min, to 200 °C at 25 °C/min and 270 °C at 10 °C/min for 7 min. The inlet temperature was kept constant at 260 °C. The mass range was 29–450 *m/z*. Peak assignments were made based on retention times and mass spectra. Inositol was added as an internal standard.

2.6. Gel permeation chromatography

Gel permeation chromatography was performed according to the method of Manrique and Lajolo (2004) with minor modification. Samples were loaded on 1 × 80 cm column packed with Sepharose 4B resin, and then eluted with 40 mM sodium acetate buffer (pH 5.0) containing 0.1% NaCl and 0.02% NaN_3 at a flow rate of 0.2 ml/min. The molecular mass calibration curve was obtained using standard dextrans with mean molecular weights of 500, 70, 40 and 10 kDa (Pharmacia, Sweden). The elute was collected every 10 min and analyzed in terms of contents of total sugars and uronic acids. The profiles of the molecular mass distributions of various samples were obtained using the molecular weight calibration curve. Blue dextran with the mean molecular weight of 2000 kDa and glucose were used to test the void volume (V_0) and the total volume (V_t) of the column, respectively.

2.7. Measurement of total sugar content

Total sugar contents were determined by the method of Dubois, Gilles, Hamilton, Rebers, and Smith (1956). Briefly, 0.5 ml of sample was incubated for 30 min with 0.5 ml of 5% (w/v) phenol and 2.5 ml of 98% (v/v) H_2SO_4. The absorbance at 490 nm was recorded. The absorbance values at 490 nm can indicate the relative content of the total sugars.

2.8. Measurement of uronic acid content

Uronic acid contents were measured by the methods of McComb and McCready (1952), with slight modification. Sample (0.5 ml) was ice-cooled and then added to 2.5 ml of 98% (v/v) H_2SO_4. The mixture solution was heated for 10 min at 100 °C. After cooling by flowing tap water, 0.25 ml of 0.15% (w/v) carbazole was added to the mixture solution. The absorbance at 530 nm was recorded after 10 min of reaction and was then used as an indicator of the relative content of uronic acid.

2.9. Evaluation of firmness of banana pulp

Ten individual fruit were used to measure fruit firmness. Peel tissues from one side of the banana finger were removed and measurements taken at three different points using a penetrometer (Model GY-1, Hangzhou Scientific Instruments, Hangzhou, China) fitted with a 4 mm diameter flat probe and recorded as Newton (N).

2.10. Data handling

Experiments were arranged in a completely randomized design. The data were analyzed by SPSS (Version 13.0). One way analysis of variance (ANOVA) and Tukey multiple comparisons were carried out to test any significant difference between the means.

3. Results and discussion

3.1. Firmness and pectin polysaccharide content of banana fruit at various ripening stages

Banana fruit is characterized by rapid softening once ripening is initiated (Jiang et al., 1999; Wills et al., 1990). In this study, fruit firmness of banana decreased rapidly from the initial 11.6 to 1.8 N after 20 days of storage at 25 °C (Fig. 1). Fruit softening is generally attributed to cellular wall disassembly, particularly due to solubilisation and depolymerization of pectin (Brummell & Harpster, 2001; Huber, 1983; Lohani, Trivedi, & Nath, 2004). As shown in Fig. 2, ASP content decreased significantly while WSP content increased from fruit ripening stages I–V, indicating that the depolymerization and solubilisation might occur. A number of reports showed the conversion of water-insoluble pectin to water-soluble pectin, similar to the results in this study, during fruit ripening (Majumder & Mazumdar, 2002; Manrique & Lajolo, 2004; Reinders & Their, 1999; Yashoda, Prabha, & Tharanathan, 2005).

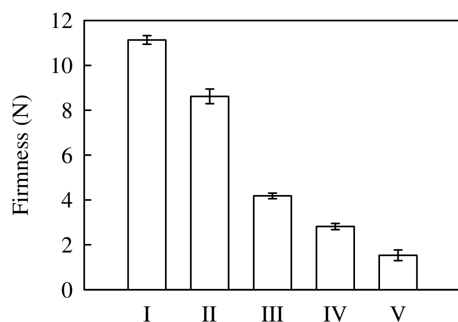

Fig. 1. Firmness of banana fruit at various ripening stages (I, mature green; II, green; III, green > yellow; IV, yellow > green and V, yellow). Data were presented as the means ± standard errors ($n = 3$).

3.2. Analyses of neutral sugar and galacturonic acid

Fig. 3 shows the molar percentage of neutral sugar and galacturonic acid of the WSP fraction after acid hydrolysis. The WSP fraction of banana pulp tissues is rich in galactose, galacturonic acid, mannose and fructose. It was determined more than 10% (molar basis) of arabinose in WSP but little amount of rhamnose and xylose was observed. During banana fruit ripening, fructose content increased but galactose and mannose contents decreased markedly. Some work reported that fructose content increased significantly in edible banana pulp during fruit ripening while the WSP degradation could contribute to the increase in the fructose content (Prabha & Bhagyalakshmi, 1998; Tarkosova & Copikova, 2000). In this study, the relative increase in fructose content may account for the decrease in galactose and mannose contents as the total percentage is constant. Similarly, this release of galactose from pectin fraction and/or the decreased pectin level have been reported during ripening of tomato (Redgwell, Fischer, Kendall, & MacRae, 1997), strawberry (Koh & Melton, 2002), papaya (Manrique & Lajolo, 2004), apple (Scalzo et al., 2005) and peach (Manganaris et al., 2006), which might be related to rapid metabolism and utilization in glycolysis (Manrique & Lajolo, 2004). The molar percentage of galacturonic acid as the main component of the backbone increased markedly as fruit ripening progressed, probably due to the removal of some of the side chains. Similar increase in uronic acid levels from the WSP fraction in cellular walls was observed in papaya (Manrique & Lajolo, 2004).

For ASP, the highest monosaccharide was galactose, followed by galacturonic acid, mannose and arabinose (Fig. 3). Small amount of rhamnose, xylose and fructose were detected in the ASP fraction. Galacturonic acid levels from ASP fraction tended to decrease whereas the proportion of galactose increased at fruit ripening stage V, which was possibly due to the consequence of a gradual breakdown of the galacturonan backbone. However, other pectic neutral monosaccharides including arabinose, rhamnose, xylose and mannose did not change significantly during fruit ripening.

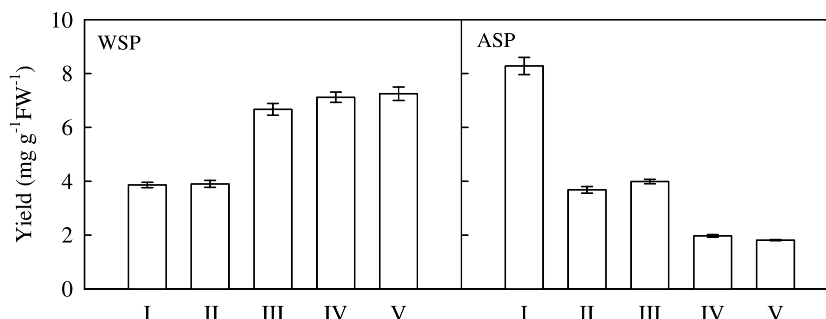

Fig. 2. WSP and ASP contents from pulp tissues of banana fruit at various ripening stages (I, mature green; II, green; III, green > yellow; IV, yellow > green and V, yellow). WSP and ASP contents were expressed as the yields on fresh weight (FW) basis. Data were presented as the means ± standard errors ($n = 3$).

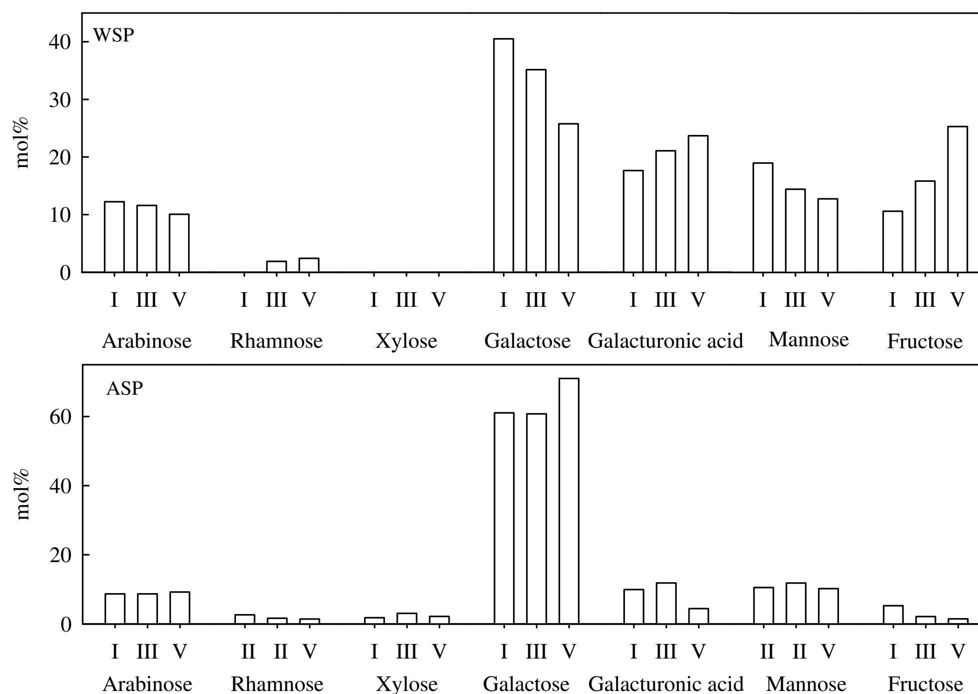

Fig. 3. Monosaccharide compositions of WSP and ASP from pulp tissues of banana fruit at various ripening stages (I, mature green; III, green > yellow and V, yellow).

Similar results were obtained in papaya and apple fruit during ripening (Manrique & Lajolo, 2004; Scalzo et al., 2005).

As discussed above, the difference in variation of monosaccharide compositions occurred in WSP and ASP fractions during fruit ripening. In this study, the WSP degradation could contribute greatly to deploymerization of side chains while the breakdown of main chains could occur mainly in ASP fraction, which may account for the different variations of monosaccharide compositions in WSP and ASP fractions.

3.3. Glycosyl linkage analysis

The fully methylated WSP and ASP fractions from banana fruit at various ripening stages were hydrolyzed with acid, then converted into alditol acetates, and finally analyzed by GC–MS. The structure-linkage characteristic of individual polysaccharides can be estimated by the molar percentage of different glycosyl residues (Smith & Harris, 1995). The study indicated that 1,4-linked Gal (2,3,6-Me_3-Gal*p*) was the major component of the backbone structure in both WSP and ASP fractions (Tables 1 and 2), which could be derived from galacturonan/galactan. However, different types of branching linkages existed in WSP and ASP fractions. In addition, all the glucose (2,3,4,6-Me_4-Glc*p*) and portions of galactose (2,3,4,6-Me_4-Gal*p*) and mannose (2,3,4,6-Me_4-Man*p*) constituted the terminal units of the WSP fraction, whereas the non-reducing terminal sugars in the ASP fraction were determined to be 2,3,4,6-Me_4-Gal*p*, 1,2,4,6-Me_4-Gal*p* and 1,2,3-Me_3-Ara*f*. Furthermore, the ratio of main chain structure (1,4-linked Gal) in WSP fraction was relatively higher than that in ASP fraction, indicating the removal of branching chains during the transformation from ASP to WSP fractions.

The most remarked change in the compositions of the ASP fraction of banana fruit during ripening is confirmed by the decrease in the amount of 1,4-linked Gal*p* residues from 58.6% in mature green fruit to 47.9% in yellow fruit on the molar basis (Table 2). Concomitantly, 1,3,6-linked Gal*p*, 1,2-linked Man*p* and 4-linked Ara*p* residues disappeared of banana fruit at the yellow stage. The loss of backbone structure in the ASP fraction could be a crucial step to result in fruit softening (Duan et al., 2007). Contrarily to the ASP fraction, the 1,4-linked Gal*p* residues in the WSP fraction exhibited a tendency of increase, probably resulting from depolymerization of some of the side chain branch-off residues during fruit ripening.

Table 1
Glycosyl linkage compositions of WSP from pulp tissues of banana fruit at ripening stages I and V

Deduced glycosyl linkages		Mol%		Sugar derivatives
		I	V	
Rhamnose	1,3-	Not detected	1.62	2,4-Me_2-Rha*f*
Mannose	1-	3.96	2.92	2,3,4,6-Me_4-Man*p*
	1,2-	10.84	19.84	3,4,6-Me_3-Man*p*
Glucose	1-	3.61	2.63	2,3,4,6-Me_4-Glc*p*
Galactose	1-	3.02	3.50	2,3,4,6-Me_4-Gal*p*
	1,6-	10.79	2.66	2,3,4-Me_3-Gal*p*
	1,4-	61.75	65.71	2,3,6-Me_3-Gal*p*
	1,3,4-	1.12	Not detected	2,6-Me_2-Gal*p*
	1,2,6-	4.92	1.13	3,4-Me_2-Gal*p*

2,4-Me_2-Rha*f* represented 2,4-di-*O*-methyl-1,3,5-tri-*O*-acetyl rhamnitol, etc. while *f* represented furanose and *p* represented pyranose.

Table 2
Glycosyl linkage compositions of ASP from pulp tissues of banana fruit at ripening stages I and V

Deduced glycosyl linkages		Mol (%)		Sugar derivatives
		I	V	
Arabnose	4-	1.04	Not detected	1,2,3-Me_3-Ara*f*
Rhamnose	1,3-	2.13	4.36	2,4-Me_2-Rha*p*
Mannose	1,2-	0.85	Not detected	3,4,6-Me_3-Man*p*
Glucose	1,6-	1.50	1.63	2,3,4-Me_3-Glc*p*
	1,3,4-	1.71	1.45	2,6-Me_2-Glc*p*
Galacose	1-	28.23	34.25	2,3,4,6-Me_4-Gal*p*
	3-	2.30	7.31	1,2,4,6-Me_4-Gal*p*
	1,4-	58.65	47.91	2,3,6-Me_3-Gal*p*
	1,2,6-	0.54	3.09	3,4-Me_2-Gal*p*
	1,3,6-	3.03	Not detected	2,4-Me_2-Gal*p*

1,2,3-Me_3-Ara*f* represented 1,2,3-tri-*O*-methyl-4,5-di-*O*-acetyl arabinitol, etc. while *f* represented furanose and *p* represented pyranose.

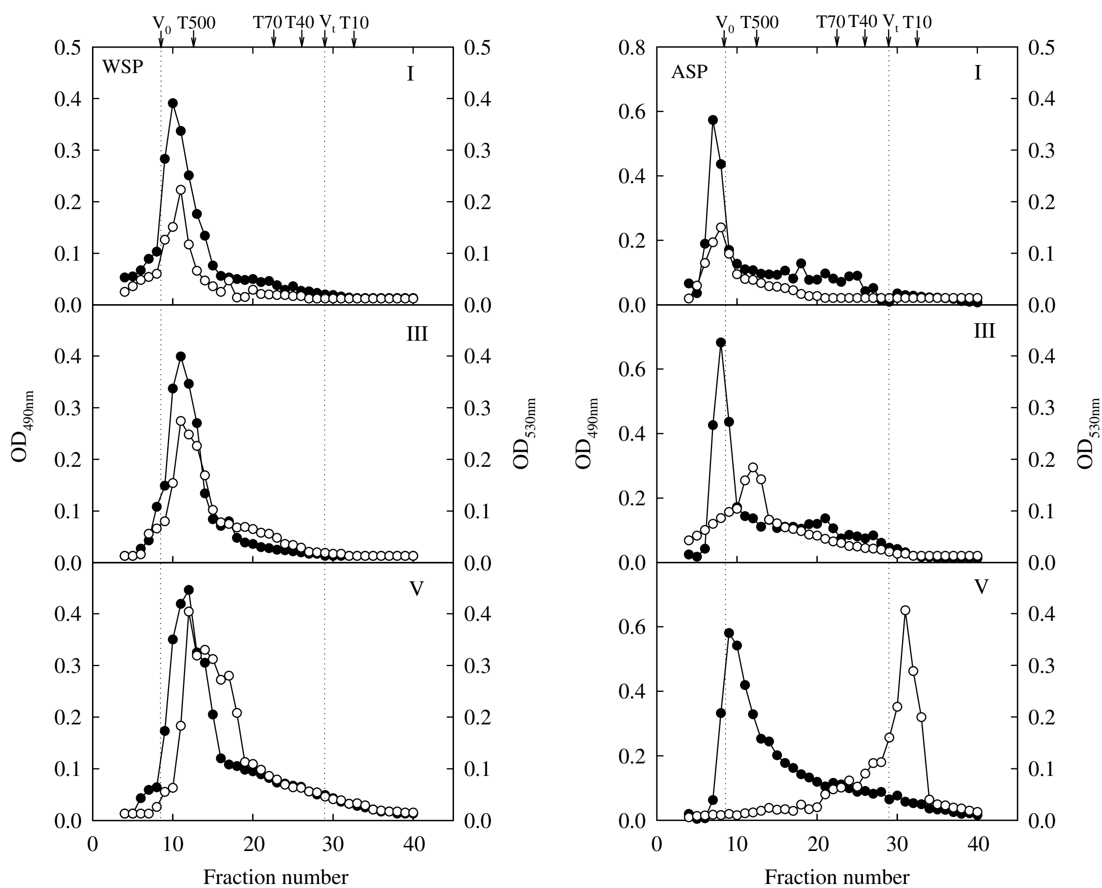

Fig. 4. Molecular mass distribution of WSP and ASP from pulp tissues of banana fruit at various ripening stages (I, mature green; III, green > yellow and V, yellow). (●) Relative content of total sugars and (○) relative content of uronic acid.

3.4. Molecular mass profile

Gel permeation chromatography was used for evaluating the molecular mass distribution of the WSP and ASP fractions from pulp tissues of banana fruit at different ripening stages. After elution, these WSP and ASP fractions were assayed for relative contents of uronic acid and total sugars. Molecular mass profiles of WSP and ASP fractions from fruit stages I, III and V were shown in Fig. 4. At stages I, III and V, the total sugar peak eluted of WSP was corresponding to an average molecular weight of approximately 819, 674 and 555 kDa, respectively using dextrans as standards, indicating further the involvement of depolymerization of WSP fraction (Fig. 4). The eluted profile of uronic acid in the WSP fraction resembled that of the total sugars except for a trailing peak at stage V. Compared with that of the WSP fraction, the total sugar profile of the ASP fraction (Fig. 4) showed a similar tendency. The molecular mass of the ASP fraction decreased gradually as fruit ripened. At ripening stages I, III and V, the average molecular masses were determined to be about 1467, 1208 and 994 kDa, respectively. Compared with the WSP fraction, uronic acid in the ASP fraction exhibited more striking molecular mass downshift from 1467 kDa at ripening stage I to 994 kDa at ripening stage V, whereas the relative uronic acid content increased rapidly when fruit ripened, implying that polyuronic acid was disassembled to oliguronic acid. This study supported further the downshifts in molecular mass distributions of pectic fractions and the depolymerisation of polysaccharides during fruit ripening/softening in pears (Hiwasa et al., 2004; Murayama, Katsumata, Endou, Fukushima, & Sakurai, 2006), avocado (Wakabayashi, Chun, & Huber, 2000), papaya (Manrique & Lajolo, 2004; Paull, Gross, & Qui, 1999) and grape (Yakushiji, Sakurai, & Morinaga, 2001).

In conclusion, the remarked changes in the composition, glycosyl linkage and molecular mass of the two pectin fractions of banana fruit occurred during ripening. As fruit softened, the molecular mass distribution of pectin polysaccharides tended to downshift. Furthermore, 1,4-linked Gal*p* residues as the major component of the backbone structure of cellular walls decreased markedly. These changes were associated with the depolymerization of pectin polysaccharides. Overall, the study indicated that modifications in polysaccharide compositions and glycosyl linkages, reduced molecular mass distributions and enhanced depolymerization of pectin during banana ripening were responsible for fruit softening.

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