Available online at www.sciencedirect.com
SCIENCE DIRECT®
Plant Science 167 (2004) 317–327
PLANT SCIENCE
www.elsevier.com/locate/plantsci

A comparative study on wall degrading enzymes, pectin modifications and softening during ripening of selected tropical fruits

Zainon Mohd Ali, Lieng-Hong Chin, Hamid Lazan*

School of BioSciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia

Received 25 November 2003; received in revised form 25 March 2004; accepted 29 March 2004

Available online 6 May 2004

Abstract

Cell wall modifying capacity and enzyme composition of rapid and slow-softening tropical fruits during ripening were investigated with respect to that of tomato, noted to contain significant endo-polygalacturonases (endo-PG; EC 3.2.1.15) activities. Besides PG, other enzymes selected to represent their pectin and/or hemicellulose modifying potentials were pectin methylesterase (EC 3.2.1.11), β-galactosidase (EC 3.2.1.23), and (1→4)-β-glucanase (EC 3.2.1.4). Time for 50% firmness loss was about 1.5 days for Beaumont guava (*Psidium guajava* L.), 3 days for tomato (*Lycopersicon esculentum* Mill.), Mas banana (*Musa acuminata*, AA group) and Eksotika papaya (*Carica papaya* L.), 4.5 days for Harumanis mango (*Mangifera indica* L.), and 20 and 24 days for B10 carambola (*Averrhoa carambola* L.) and Kampuchea guava, respectively. Capacity to markedly modify cell walls as reflected by capability to impact increased pectin solubility and depolymerization of chelator-soluble pectin, and which varied markedly with the fruit types, may not necessarily be correlated with softening rate, neither was it dependent on the presence of any significant endo-PG activities. Extensive pectin modifications occurred in ripening tomato, mango and papaya. Beaumont guava and Mas banana, though softened rapidly, experienced much limited pectin degradation, seemingly unable to match the modifications that occur in the slow-softening carambola and Kampuchea guava. All fruits tested, except tomato, contained significant exo-PG (EC 3.2.1.67) activities, thus explaining that endo-PG might not be the determining factor of differential softening. All other enzymes, viz. pectin methylesterase, (1→4)-β-glucanase, and in particular, β-galactosidase seemed relevant and might contribute significantly to the observed variations in softening rate amongst the fruits.

Keywords: Tropical fruits; Banana; Carambola; Guava; Mango; Papaya; Tomato; Softening enzymes; Pectin modifications; Ripening

1. Introduction

Tropical fruits such as mango (*Mangifera indica* L., family Anacardiaceae), papaya (*Carica papaya* L., family Caricaceae), banana (*Musa acuminata*, AA group, family Musaceae), carambola (*Averrhoa carambola* L., family Oxalidaceae), and guava (*Psidium guajava* L., family Myrtaceae), were reported to contain markedly different levels of structural and non-structural carbohydrates, apart from the possibility that these fruits differed markedly in their softening rates during ripening and storage life [1–3]. With respect to the non-structural carbohydrates, mature fruits of banana and mango contain as high as 20–25% on fresh weight basis of starch, while guava, papaya, and starfruit contain either very little or no discernible amounts of starch [4–7]. Although starch content differed markedly with the fruit type, these differences are not always reflected in their firmness levels at maturity or their softening rates during ripening [8,9]. From a horticultural perspective, tissue firmness is an important quality attribute and the rate of firmness loss during ripening may influence not only fruit quality but also its storage life.

The mechanisms by which fruits soften during ripening remain unclear and are subject to much speculation. Although turgor loss and starch degradation and subsequent decline in its content during ripening might contribute, however, enzyme-catalyzed changes to wall structure and composition are considered the major factor of softening of fruits [1,10–13]. Despite the likely possibility that fruits contain more or less similar make up of wall enzymes, the manner the various cell wall carbohydrate components are modified suggests that there are subtle regulatory mechanisms at the

* Corresponding author. Tel.: +60-3-8921-5869; fax: +60-3-8925-2689.
E-mail address: hlazan@pkrisc.cc.ukm.my (H. Lazan).

0168-9452/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.plantsci.2004.03.030

level of enzyme concentration, the type of enzyme isoforms present, and the timing of appearance of those different isoforms might be important in orchestrating wall disassembly and fruit softening [14–18]. In general, all cell wall components, viz. pectins, non-cellulosic cross-linking glycans (previously known as the hemicelluloses), and cellulose might be modified during ripening, but the timing, speed, and extent of their enzyme-catalyzed modifications vary markedly with the fruit type [2,3,19–26]. Besides enzymes functioning as catalysts for wall modifications, differences in the architecture of the primary walls between fruits may also contribute to differences in their softening rate [27].

In the present study, tropical fruits or fruit cultivars were selected to represent slow- and rapid-softening fruit types and the biochemical bases of the differences in their softening rates were compared with that of tomato, considered to be the most extensively studied fruit with respect to softening mechanisms and wall pectin modifications, apart from containing high levels of endo-polygalacturonase (PG) activities [10,28]. It was apparent from analysis on transgenic tomato expressing anti-sense PG gene, the wall enzyme might not be the crucial factor in affecting cell wall disassembly and softening of fruits [10,13,20]. Other enzymes such as endo-glycanases which include, among others, (1→4)-β-glucanase and β-galactanase and functional proteins expansins might be important in impacting major structural changes to the wall early during ripening and necessary to effect softening [1,12,13,29]. The significance of these wall enzymes as well as PG and pectin methylesterase (PME) to variations in the softening rate during ripening of papaya, mango, banana, guava, and carambola fruits was investigated.

2. Materials and methods

2.1. Plant materials, firmness determination, and tissue sampling

Mature, unripe guava (*Psidium guajava* L. cvs. Beaumont and Kampuchea), tomato (*Lycopersicon esculentum* Mill. cvs. MT11, Intan RK, and AV4), banana (*Musa acuminata* cv. Mas, AA), papaya (*Carica papaya* L. cv. Eksotika), mango (*Mangifera indica* L. cv. Harumanis), and carambola (*Averrhoa carambola* L. cv. B10) fruits were harvested from private farms in the states of Selangor and Perlis, Malaysia. The fruits were washed with water, soaked in fungicide (0.02% prochloraz, 5 min), and left to ripen at ambient temperature (ca. 28 °C). Five to six fruits from each species were sampled at harvest date and at full ripeness for biochemical and cell wall analyses. For firmness determination, measurements were made on 10 fruits at regular intervals through ripening using a McCormick Pressure Tester (model FT327-12, Milan, Italy). Procedures for firmness estimation and tissue sampling for biochemical analyses of carambola, mango and papaya, were similar to the method as described previously [3,30,31]. Briefly, for carambola, firmness measurements were made on three sites on any one of the fruit's wings, while for mango and papaya determinations were made on the cut surface located on the middle section of the fruits. For banana, firmness readings were made on three regions on the midsection, while for tomato and guava measurements were made on the equatorial sector of the fruits. For tissue sampling, pulp tissue at the midsection of banana and entire mesocarp of tomato and guava were used. All tissue samples were kept at −70 °C until required for analysis.

2.2. Enzymes extraction and assay

Tissues (ca. 10 g) were homogenized in blender (Edmund Bühler 7400, Tübingen, Germany) in 20 ml cold (4 °C) 0.1 M sodium citrate, pH 4.6, containing 1 M NaCl, 13 mM EDTA, 10 mM β-mercaptoethanol, and 1% (w/v) PVP-40. The supernatant was recovered by centrifugation (Sorvall RC-5B Superspeed, 29,000 × *g*, 30 min), filtered through a double layer nylon cloth, and an aliquot (2 ml) desalted on a Sephadex G-25 (1 × 10 cm) column prior to assay for enzymes activities except pectin methylesterase. To assay for PME, undesalted fruit extract was used. Polygalacturonase, β-galactosidase and PME were assayed according to the procedures as detailed earlier [31,24]. (1→4)-β-Glucanase was assayed using CM-cellulose as a substrate essentially as described previously [3] and the amount of reducing sugar released determined using the DNS (3,5-dinitrosalicylate) reagent [32]. Endo-PG activity was estimated by measuring the specific viscosity loss and the amount of reducing sugar formed [28]. Briefly, assay mixture comprised 7.5 ml of 1.5% (w/v) polygalacturonic acid, pH 5.2, 1 ml 0.6 M NaCl, one to two drops of toluene, 1.5 or 3 ml desalted extract of ripe fruits and extracting buffer to volume, and flow times were measured using a Euroglas viscometer (Büiten Watersloot 341 Delft, Holland) at regular intervals for the first 3 h and subsequently after 7, 10, 24, 34, 48 and 50 h of incubation at 37 °C. For PG activities, reducing sugar released was estimated by the cyanoacetamide method [33].

2.3. Extraction and analysis of pectin

Procedures for the preparation of alcohol-insoluble solids (AIS) and for the extraction and analysis of chelator-soluble pectins (CSP) were as described previously [31]. Briefly, the AIS wall material was suspended (37 °C, 8 h) in 80 mM sodium acetate buffer, pH 5, containing 10 mM EDTA. The supernatant was recovered and its uronic acid and carbohydrate contents determined using the methods as described elsewhere [34,35]. Total polyuronides in the AIS was also estimated [19]. Gel filtration chromatography of the CSP (ca. 1.5 mg uronic acid in 30 mM sodium acetate, pH 5, containing 10 mM EDTA) was carried out on a Sephacryl S-500 HR (Pharmacia, Sweden) column (1.6 cm × 54 cm). Fractions (2.5 ml) were collected by eluting with the buffer

containing 50 mM NaCl at a flow rate 7.2 ml h^{-1} and analyzed for their uronic acid and carbohydrate contents.

3. Results

3.1. Fruit softening and pectin modification

Tissue firmness at commercial maturity stage, and firmness loss rate varied markedly with the fruit types (Fig. 1). Beaumont and Kampuchea guavas, Eksotika papaya, and Harumanis mango were significantly firmer than tomato, Mas banana, and B10 carambola with values ranging from 100–120 to 30–60 N, respectively (Fig. 1a). Beaumont guava had the fastest softening rate with time for 50% firmness loss of about 1.5, followed by 3.0 in tomato, banana and papaya, and 4.5 days in mango (Fig. 1b). Carambola and Kampuchea guava were slow softeners, with time for 50% firmness loss of about 20 and 24 days, respectively. Besides softening, and with the exception of Harumanis mango that remained green, ripening of the other fruits was also characterized by changes in skin color (data not shown). Based on fruit firmness and color, there was a 5–24-day interval between harvest date and full ripe stage among the fruits. Beaumont guava, tomato, and Mas banana took about 5 days to reach full ripeness, compared with 8–10 days for papaya and mango, and 24 days or longer for carambola and Kampuchea guava (Table 1, Fig. 1).

Pectin levels measured as total polyuronides as well as chelator-soluble polyuronides and carbohydrates in unripe mature fruits varied markedly (Table 1). Total polyuronide levels were relatively high in tomato and mango (500–400 mg g^{-1} AIS), moderate in papaya, banana, and carambola (350–250 mg g^{-1} AIS) and low in guavas (160–120 mg g^{-1} AIS). However, the amount of soluble polyuronides present as percentage of total in unripe fruits did not correlate with their total polyuronide contents. Less than 10% of the total polyuronides were in soluble form in banana and mango, compared with 20–30% in carambola, tomato, papaya and Beaumont guava, and about 50% in Kampuchea guava (Table 1).

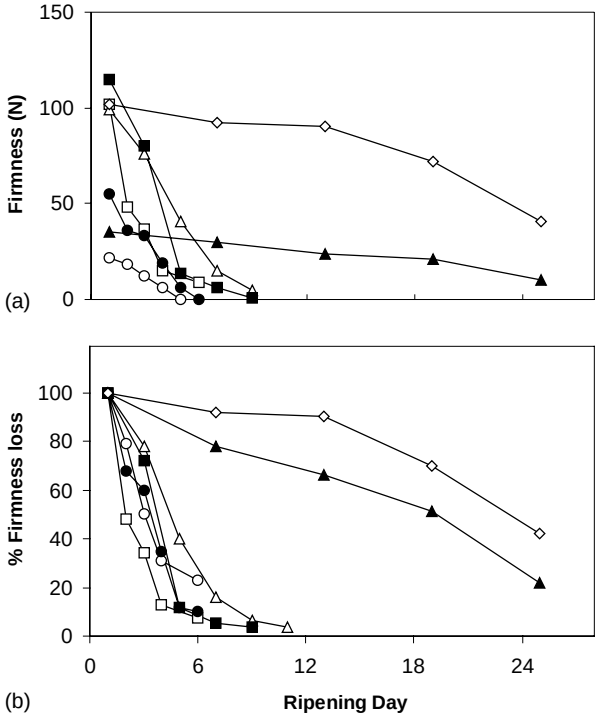

Fig. 1. Firmness changes (a) and differential firmness loss rates (b) during ripening of Beaumont guava (□), tomato (○), banana (●), papaya (■), mango (△), carambola (▲), and Kampuchea guava (◇) fruits. Results are means of five fruits ± standard errors.

During ripening, total pectin content in all fruits, except Kampuchea guava, declined while pectins were being increasingly solubilized as reflected by increase in soluble polyuronide and carbohydrate levels (Table 1). Pectin solubilization was substantial in mango and banana, registering about 340 and 272% increase in their soluble polyuronide levels, respectively, during ripening. Significant solubilization was noted in tomato (143%), papaya (108%), and carambola (92%), and moderate in Kampuchea (45%) and Beaumont (31%) guavas. In mango, increased pectin solubility was accompanied by a substantial 130% loss in total polyuronides, while in the other fruits, viz. tomato (15%), carambola (19%), banana (27%), papaya (34%),

Table 1
Ripening times and levels of total polyuronides and of chelator-soluble polyuronides and carbohydrates (mg g^{-1} AIS) in ripening tropical fruits

Fruit type	Time to full ripe (days)	Total polyuronides		Chelator-soluble polyuronides		Chelator-soluble carbohydrates	
		Mature green	Ripe	Mature green	Ripe	Mature green	Ripe
Guava cv. Beaumont	5	155 ± 21	90 ± 9	48 ± 8	63 ± 6 (31)	37 ± 2	64 ± 5 (73)
Tomato cv. MT11	5	516 ± 23	438 ± 25	138 ± 13	336 ± 23 (143)	122 ± 6	237 ± 6 (94)
Banana cv. Mas	5	296 ± 16	216 ± 20	25 ± 2	93 ± 7 (272)	26 ± 2	80 ± 4 (208)
Papaya cv. Eksotika	8	326 ± 15	215 ± 9	89 ± 1	185 ± 2 (108)	64 ± 1	180 ± 3 (181)
Mango cv. Harumanis	10	429 ± 53	294 ± 28	38 ± 2	167 ± 2 (340)	57 ± 1	190 ± 1 (233)
Carambola cv. B10	24	250 ± 14	202 ± 28	50 ± 4	96 ± 8 (92)	67 ± 5	112 ± 8 (67)
Guava cv. Kampuchea	>24[a]	119 ± 15	120 ± 13	56 ± 6	81 ± 4 (45)	51 ± 6	72 ± 10 (41)

Values are mean of six fruits ± standard errors. Figures in brackets are percent increase in pectin solubility through ripening.
[a] Day when experiments were stopped.

and Beaumont guava (42%) only moderate losses were registered. Exception was Kampuchea guava that showed no pectin losses (Table 1). Indeed, in this fruit, increased pectin solubility and firmness loss during an extended ripening period was accompanied by increased pectin level [8], perhaps suggesting that pectin is being continuously synthesized throughout development of the fruit.

Analysis of molecular size distribution by gel filtration on Sephacryl S-500 HR column of chelator-soluble polyuronides and carbohydrates were performed on unripe and ripe fruits, and results are as shown in Figs. 2 and 3. Greatest ripening-related pectin downsizing capacity particularly with respect to the polyuronides and to a certain extent also the neutral carbohydrates, in terms of both magnitude and speed, in descending order, was recorded in tomato, mango, and papaya (Figs. 2b,d and e and 3b,d and e). Quite significant depolymerization but at a much slower rate (over 24 days) was registered in carambola (Figs. 2f and 3f), the extent of which was markedly greater than that occurring in the very rapid-softening Beaumont guava (Figs. 2a and 3a) or in the very slow-softening Kampuchea guava (Figs. 2g and 3g). Both guava cultivars, however, seemed to have comparable pectin modification potentials, differed significantly in terms of their pectin downsizing and firmness loss speeds (Figs. 1–3). The rapidly softening banana was unique; though pectin solubility increased substantially, however, no discernible pectin depolymerization was observed during ripening (Table 1; Figs. 2c and 3c).

3.2. Polygalacturonase activity

Table 2 shows varying levels of PG activities in unripe and ripe fruit tissues of tomato and the tropical fruits studied. The enzyme activity in unripe tissues was comparable regardless of the fruit types ranging from 1.3 to 2.7 nkat g^{-1} FW, and increased with ripening in all fruits. The greatest, about 500% increase in PG activity, was recorded in tomato compared to about 300 and 250% increase in papaya and banana, respectively. These fruits, incidentally, recorded highest PG activity levels in ripe tissues ranging from about 6 in papaya to about 8 and 10 nkat g^{-1} FW in tomato and banana, respectively. The very rapid- and slow-softening Beaumont guava and carambola, respectively, had comparable PG levels and registered a small 150% increase compared to their initial activity of about 1.4 nkat g^{-1} FW. The slow-softening Kampuchea guava registered only a discernible 20% increase in PG activity, almost comparable with the increase recorded in rapid-softening mango.

The identity of the major form of PG in the different fruit types was determined by measuring the loss of specific viscosity of polygalacturonic acid (PGA, 1.5%, w/w) substrate and the concomitant increase in reducing sugar being hydrolyzed, assayed over a 50 h period using ripe fruit extracts. Typical results for three different tomato cultivars viz. Intan RK, MT11 and AV4, which exhibited a rapid decline in substrate specific viscosity and accompanied by a rapid

Table 2
Polygalacturonase, pectin methylesterase, β-galactosidase, and (1→4)-β-glucanase activities in ripening tropical fruits

Fruit type	Polygalacturonase (nkatal g^{-1} FW)		Pectin methylesterase (nequiv. s^{-1} g^{-1} FW)		β-Galactosidase (nkatal g^{-1} FW)		(1→4)-β-Glucanase (nkatal g^{-1}FW)	
	Mature green	Ripe	Mature green	Ripe	Mature green	Ripe	Mature green	Ripe
Guava cv. Beaumont	1.4 ± 0.3	3.7 ± 0.5 (164)	12 ± 0.2	21 ± 2 (81)	4.0 ± 0.2	7.0 ± 0.7 (75)	25 ± 2.2	25 ± 3.2 (0)
Tomato cv. MT11	1.3 ± 0.2	7.6 ± 0.8 (485)	468 ± 90	653 ± 91 (40)	3.2 ± 0.3	3.2 ± 0.2 (0)	6.4 ± 1.8	14 ± 2.0 (119)
Banana cv. Mas	2.7 ± 0.7	9.5 ± 1.7 (252)	336 ± 11	373 ± 18 (11)	7.7 ± 0.8	14 ± 1.6 (77)	8.7 ± 0.8	35 ± 2.4 (296)
Papaya cv. Eksotika	1.6 ± 0.3	6.3 ± 0.9 (294)	63 ± 13	463 ± 32 (636)	4.3 ± 0.2	16 ± 2.0 (249)	30 ± 2.3	31 ± 5.2 (3)
Mango cv. Harumanis	1.4 ± 0.4	2.2 ± 0.6 (57)	757 ± 92	313 ± 26 (−59)	3.0 ± 0.2	24 ± 5.9 (703)	7.0 ± 1.0	17 ± 0.8 (140)
Carambola cv. B10	1.3 ± 0.2	3.3 ± 0.3 (154)	29 ± 2	73 ± 6 (150)	6.7 ± 1.0	11 ± 1.2 (70)	46 ± 6.6	68 ± 7.4 (47)
Guava cv. Kampuchea	1.8 ± 0.3	2.2 ± 0.3 (22)	9 ± 1	11 ± 1 (32)	3.7 ± 0.6	4.6 ± 0.6 (24)	21 ± 2.6	32 ± 2.4 (80)

Values are mean of six fruits ± standard errors. Figures in brackets are percent increase (or decrease) in enzyme activity through ripening. Time to full ripe stage: 5 days (Beaumont guava, tomato, banana); 8 and 10 days (papaya and mango, respectively); 20 and >24 days (carambola and Kampuchea guava, respectively).

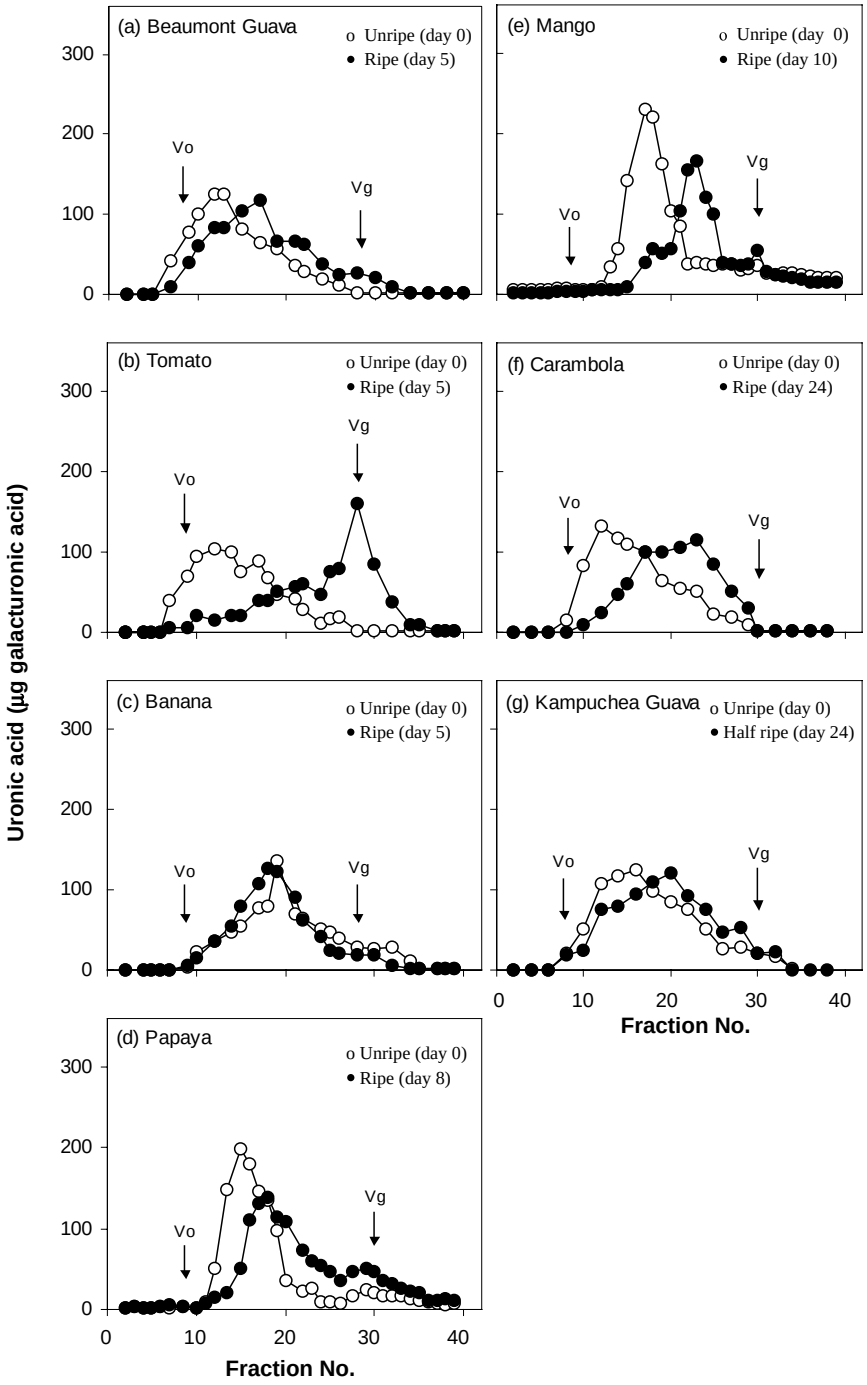

Fig. 2. Uronic acid elution profiles on Sephacryl S-500 HR column of EDTA-soluble pectin fractions from unripe (○) and ripe (●) Beaumont guava (a), tomato (b), banana (c), papaya (d), mango (e), carambola (f), and Kampuchea guava (g) fruits. Void volume, V_o, and elution volume for glucose, V_g.

hydrolysis of the glycosidic bonds are shown in Fig. 4. A 50% loss in specific viscosity with only about 1–3% of the glycosidic bonds being hydrolyzed was established within 10 h of incubation. These hydrolytic patterns are typical for endo-acting PGs. Unlike tomato, other fruits exhibited much limited substrate viscosity loss and with very little glycosidic bond hydrolysis over the same incubation period (Fig. 5a versus Fig. 5b–g). Even after a 50 h reaction, only about 1–2% of the glycosidic bonds were being hydrolyzed and a specific viscosity loss of less than 20%; these results either suggest that exo-PGs were the predominant form in all of the tropical fruits tested or that the enzyme was unstable during an extended assay time.

The possibility that endogenous inhibitors might have affected the enzyme's activity was investigated by adding equal volumes (1.5 ml) of extracts from the respective

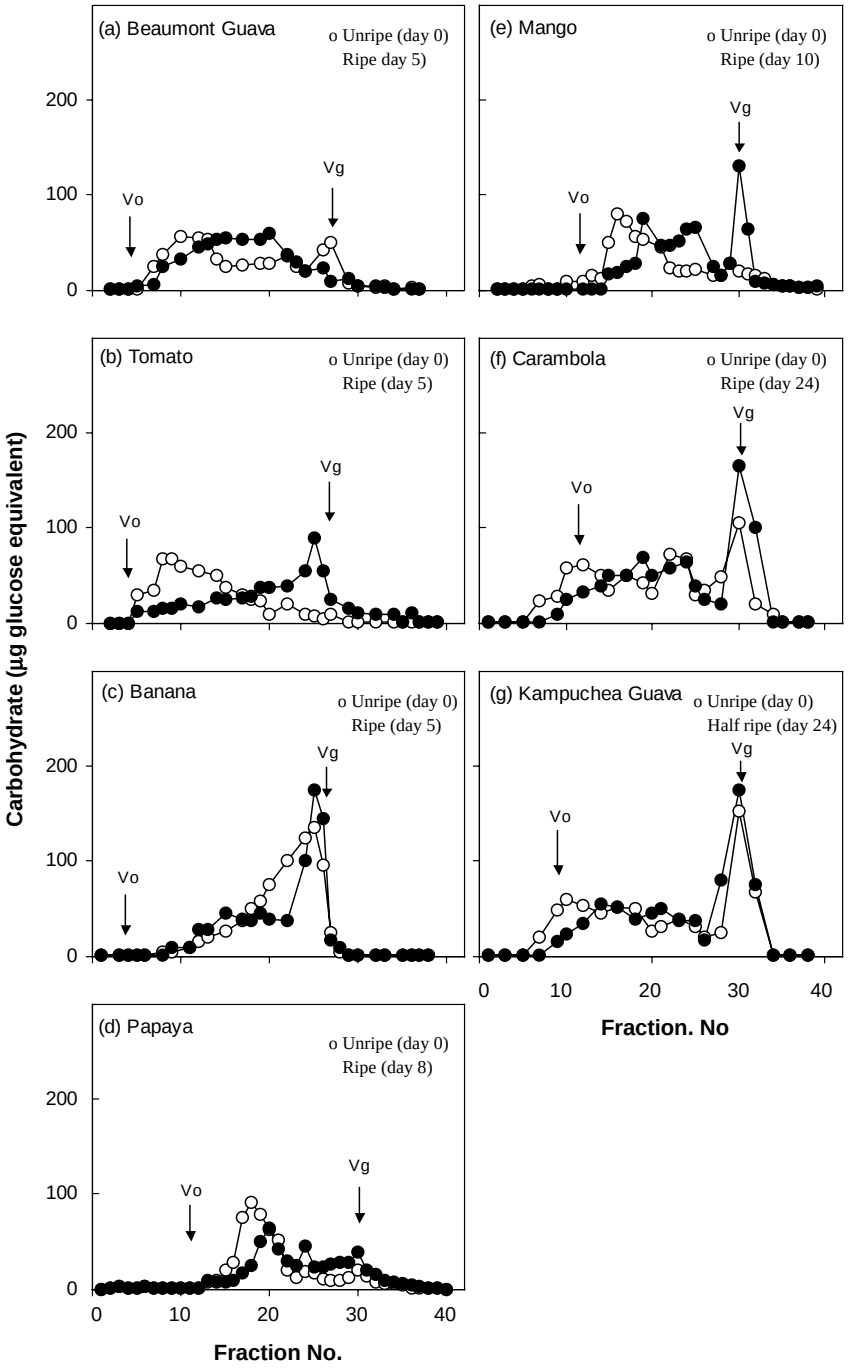

Fig. 3. Neutral carbohydrate elution profiles on Sephacryl S-500 HR column of EDTA-soluble pectin fractions from unripe (○) and ripe (●) Beaumont guava (a), tomato (b), banana (c), papaya (d), mango (e), carambola (f), and Kampuchea guava (g) fruits. Void volume, V_o, and elution volume for glucose, V_g.

tropical fruits to that of tomato in the assay mixture. Results from this extract co-incubation studies indicated that endo-PG activity of the tomato fruit was not inhibited, thus proving that the low or lack of endo-PG activity in the tropical fruits was not due to the presence of inhibitors at least of tomato PG (Fig. 5a–g). PG from all fruits also appeared to release only monogalacturonic acid (MGA) residues after 48 h reaction with 1.5% (w/v) PGA substrate as analyzed by thin layer chromatography (TLC). For tomato, besides MGA residues, TLC analysis after 48 h incubation exhibited the presence of a distinct chromatographic streak trailing the MGA front, believed to comprise the oligomeric products of endo-PG action (8). The predominant enzyme form that is present in the tropical fruits examined thus appeared to be exo-PG. Among the fruits tested, banana seemed to be different. When sample volume in the banana assay mixture

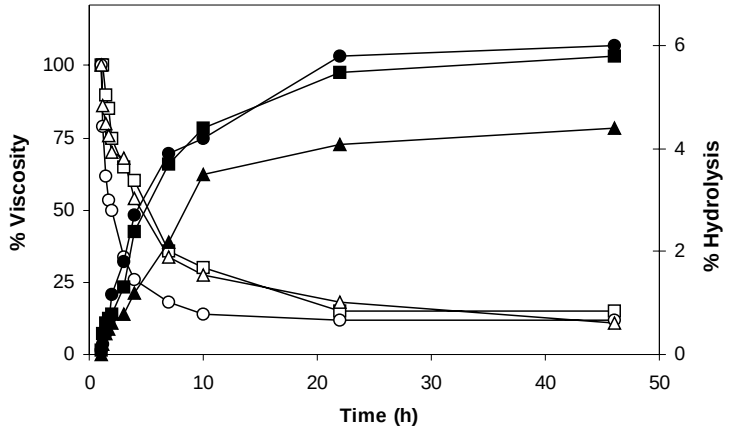

Fig. 4. Time course changes in specific viscosity (open symbols) and in glycosidic bond hydrolyzed (closed symbols) during reaction of enzymes from different cultivars of ripe tomato fruits with polygalacturonic acid substrate. Tomato cultivars MT11 (○●), Intan RK (△▲) and AV4 (□■).

was doubled (from 1.5 to 3 ml), PG activity as assayed viscometrically, increased significantly, albeit in a still exo-acting fashion (Fig. 5b versus Fig. 5a). The result suggests that banana might probably contain discernibly higher endo-PG activity apart from the possibility it also contains higher exo-PG than the other tropical fruits. Results of PG activities as assayed by the reducing sugar method and presented in Table 2 are consistent with the possibility that banana contained higher PG activity than the other fruits.

3.3. Pectin methylesterase, β-galactosidase and (1→4)-β-glucanase activities

Varying levels of pectin methylesterase, β-galactosidase, and (1→4)-β-glucanase activities were detected in mature tropical fruits (Table 2). PME activity was low in mature fruits of both guava cultivars (ca. 10 activity units), quite low in carambola and papaya (30–60 units), high is banana and tomato (300–500 units), and very high in mango (>700 units). Excepting mango that exhibited a substantial 40% decline in PME activity, the enzyme activity in all other fruits increased during ripening (Table 2). The increase was dramatic in papaya (>600%), moderate in carambola (150%), low in Kampuchea guava, tomato, and Beaumont guava (30–80%), and very low in banana.

β-Galactosidase activities in all mature fruits studied ranged from 3 to 8 units, with the higher activities found in carambola and banana. As the fruits ripened, a substantial 7-fold increase was registered in mango, followed by papaya (250%) and moderate increases (about 70%) in carambola, Beaumont guava, and banana, and a low increase in Kampuchea guava. Tomato fruit, however, showed no increase in total β-galactosidase activity during ripening, which seems typical for the fruit species [36].

(1→4)-β-Glucanase measured as carboxymethyl cellulase activity in mature fruit tissues, like PME, also varied markedly with the fruit types (Table 2). Very high activity was recorded in carambola (>45 units), quite high activities in papaya and both guava cultivars (30–20 units), and low in tomato, mango, and banana (8–6 units). The enzyme activity registered a substantial 300% increase to a relatively high level (ca. 35 units) during ripening of banana, and moderate 120–140% rise in tomato and mango, and a discernible increase in carambola and Kampuchea guava. No increase in (1→4)-β-glucanase activity was observed in ripening papaya and Beaumont guava, which already contained quite high activity levels in mature, unripe tissues.

4. Discussion

Softening rate during ripening differed markedly with the fruit type, with Beaumont and Kampuchea guavas exhibiting the fastest and the slowest rates, respectively. Other rapid-softening fruits in descending order were tomato, banana, papaya, and mango, while carambola had a moderately slow-softening speed. It seems that pectin modifications alone might not be adequate to account for the observed differential softening rates amongst the fruits. Likewise, it would be unlikely that differences in the rate be dependent upon the decline in starch content because fruits that contain very little or no starch such as guava, tomato, and papaya softened as rapid as, if not faster than, those that contain high starch levels such as banana and mango (Fig. 1a and b). It seems, besides pectin, other wall components, particularly hemicelluloses, might contribute to differences in the softening rate because hemicelluloses, too, were reportedly to be modified during ripening of these fruits [2,3,19,24,37,38].

Though all fruits studied contained significant polygalacturonase levels, this wall degrading enzyme was probably not the major factor in differential softening; these fruits except tomato, regardless of their softening rate and extent of pectin modification, appeared to contain relatively high exo-PG and low endo-PG activities (Table 2; Figs. 4 and 5). The case of transgenic tomatoes engineered to produce substantially low endo-PG levels further supports the

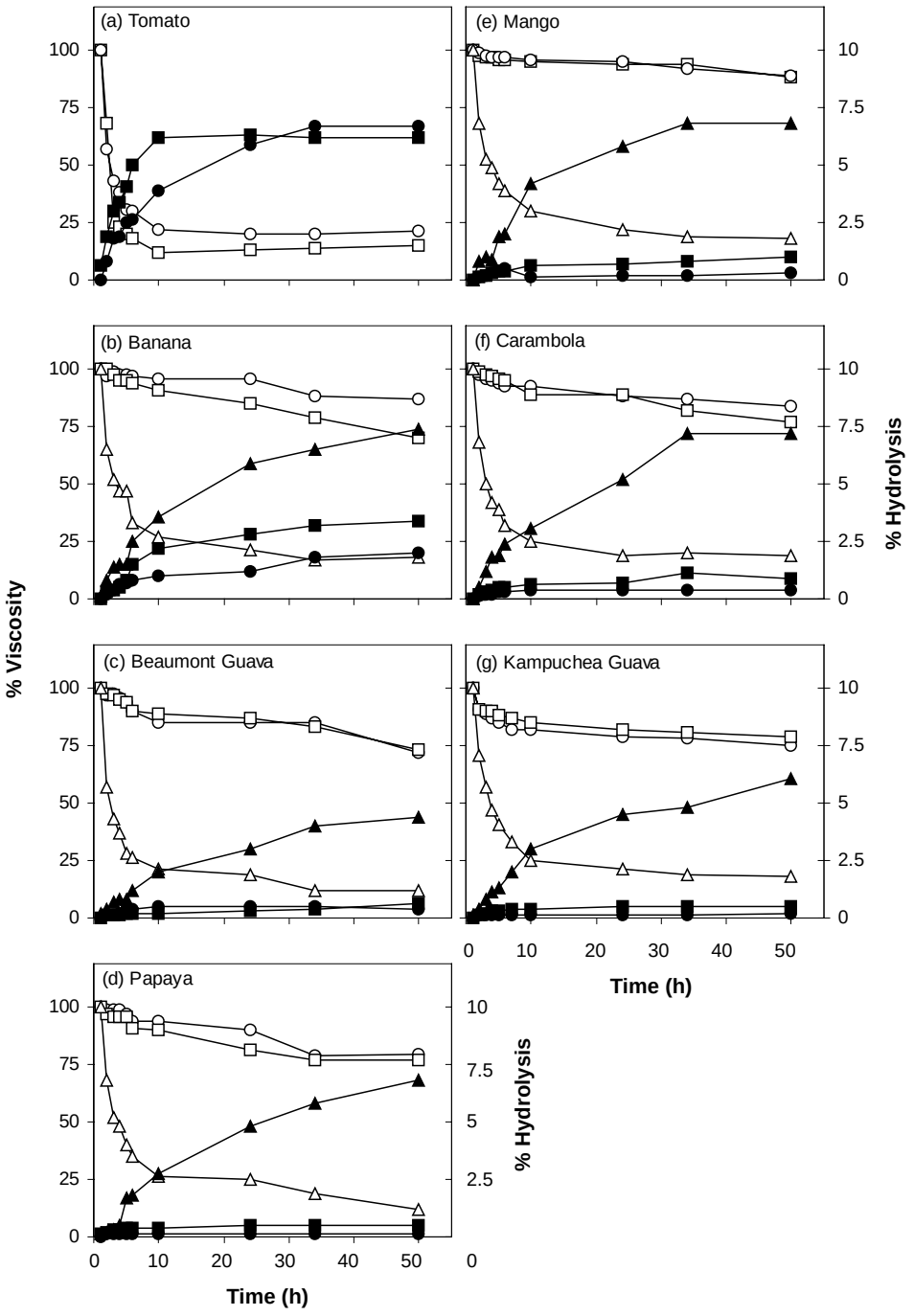

Fig. 5. Effects of varying fruit extract volume ((○●), 1.5 ml; (□■), 3 ml) and of combining equal volume (1.5 ml) of tomato and other fruit extracts (△▲) on time course changes in specific viscosity (open symbols) and in glycosidic bond hydrolysed (closed symbols) during reactions of enzyme or combination of enzymes from ripe MT11 tomato (a), banana (b), Beaumont guava (c), papaya (d), mango (e), carambola (f), and Kampuchea guava (g) fruits with polygalacturonic acid substrate.

hypothesis that PG may not be the determining factor in wall disassembly and softening of fruits [10]. Primary cell walls being complex in structure, thus, it is unlikely for any particular enzyme alone to be able to significantly modify the wall and to effect softening. A collective action of a number of enzymes acting synergistically would be needed to impact any significant texture changes.

Pectin methylesterase activities seemed significant, but its role in differential softening of the tropical fruits is unclear. Depending on the fruit type, the enzyme activity either increased markedly and/or was sustained at very high levels during ripening (Table 2). Perhaps, high pectin demethylesterification activities, as catalyzed by PME, are required not only for subsequent PG action but also to

modify pH and cation exchange properties of the wall [39], which might, in turn, affect activity of other wall degrading enzymes. Notable among the enzymes that may have significant impact on wall disassembly during ripening are the endo-glycanases such as (1→4)-β-glucanases and β-galactosidases with β-galactanase activities [13]. The latter enzyme, in particular, was reported to have the capability to simultaneously modify pectin and hemicelluloses [8,18].

Both β-galactosidase and (1→4)-β-glucanase were present, although their activity levels in ripening tissues varied markedly with the fruit types (Table 2). The fast-softening banana contained quite substantial levels of β-galactosidase and (1→4)-β-glucanase, and the enzymes activity increased markedly with ripening. Mango seemed also to depend on the increase in these enzymes activities for rapid-softening. Papaya, another rapid-softening fruit, exhibited marked increase in β-galactosidase, apart from sustained high (1→4)-β-glucanase activity levels through ripening. Likewise, the rapid-softening Beaumont guava maintained high (1→4)-β-glucanase activities through ripening, however, its β-galactosidase activity registered only a moderate increase, if compared to papaya. Carambola and Kampuchea guava also contained significant β-galactosidase and in particular, (1→4)-β-glucanase activities. However, unlike the rapid-softening types, the activity of these as well as the other wall enzymes increased gradually during ripening of these slow-softening fruits. Tomato appeared unique, though containing substantial endo-PG activity and experiencing extensive pectin modifications. The fruit exhibited relatively low levels of both β-galactosidase and (1→4)-β-glucanase activities. While (1→4)-β-glucanase activity in tomato increased, that of β-galactosidase showed no increase during ripening and similar to results as reported earlier [36].

In tomato, β-galactosidase occurs in multiple forms, possibly encoded by a multigene family. Out of the seven β-galactosidase genes reported in tomato, only one, namely that encoded a β-galactosidase 2, appeared relevant to ripening [17]. Mango, papaya, and carambola also contained a number of β-galactosidases and it seems not all of the isoforms are significant in fruit softening during ripening [8,15,40]. Of the β-galactosidase isoforms reported in these tropical as well as other fruits, a number have (1→4)-β-galactanase activities [14,36,41]. While the activity of β-galactosidase/galactanase in apple, tomato, and pear fruits were attributed to exo-acting enzyme, that in carambola and papaya appeared to be related to endo-galactanase [8,18]. Besides (1→4)-β-galactanase, (1→4)-β-glucanase as well as PG and PE were also reported to occur as distinct isoforms [16,39,42].

The functional significance of β-galactosidase/galactanase as well as (1→4)-β-glucanase to wall modifications and softening is unclear, and may conceivably depend on the precise architecture of the primary wall, which may differ markedly with the fruit types. One perceived model [43] is that pectin networks are linked covalently with the xyloglucan–cellulose microfibril complex, while another model suggests that pectin and xyloglucan–microfibril networks are architecturally independent [27]. The suggestion that β-galactosidase/galactanase might contribute significantly to wall modifications and softening implies that the enzyme or its specific isoforms act upon the purported cross-linking galactans that link the pectin rhamnogalacturonans to the xyloglucan–cellulose microfibril complex [43–45]. The (1→4)-β-glucanase would conceivably function by degrading glycan crosslinks such as the xyloglucans that tether cellulose microfibrils or it might act upon glucan polymers that reside within the amorphous regions of the microfibrils [13,23]. Pectin demethylesterification activities as catalyzed by PME might directly or indirectly assist other enzymes besides PG by creating suitable ionic environments or perhaps also, by modifying wall porosity, a physical property attributable to the matrix pectin, and thereby affecting accessibility of enzymes to their potential substrates.

It seems probable that wall enzymes may function collectively and synergistically over time and space, in impacting wall disassembly during ripening, the speed and extent of which would depend on the precise architecture of the primary wall. Enzymes with capability to catalyze the cleavage of cross-linking glycans that interconnect cellulose microfibril–xyloglucan–pectin networks such as β-galactosidase/galactanases and/or (1→4)-β-glucanases might function early during the ripening process to initiate wall disassembly. PG as well as other isoforms of the glycanases may probably come later to impart greater wall modification and softening change [13,18]. In this regard, evidence with the β-galactanases of papaya suggests that it is the composition of specific isoforms, their relative concentrations or activity levels, and the precise timing of their presence during ripening that appeared to be important, observations which might be relevant to differential softening of fruits [18].

In summary, differential softening phenomenon amongst the tropical fruits investigated cannot be attributed simply to differences in the modification of the wall pectin, neither to differences in PG activities. Instead, integrated modification of both the pectin and the xyloglucan–cellulose microfibril networks brought about by the possible action of such glycanases as β-galactosidase/galactanases and (1→4)-β-glucanases might be instrumental. Besides the glycanases, abundance of PME activity also appears relevant to extensive wall modifications and rapid-softening. The tropical fruits are unique in that, unlike tomato, they contained significant amounts of exo-PG relative to endo-PG activities.

Acknowledgements

We thank the Ministry of Science, Technology and Environment of Malaysia for their financial support under the IRPA grant 01-02-02-0027.

References

[1] H. Lazan, Z.M. Ali, Cell wall hydrolases and their potential in the manipulation of ripening of tropical fruits, ASEAN Food J. 8 (1993) 47–53.

[2] K. Kojima, N. Sakurai, S. Kuraishi, Fruit softening in banana: correlation among stress-relaxation parameters cell wall components and starch during ripening, Physiol. Plant. 90 (1994) 772–778.

[3] L.H. Chin, Z.M. Ali, H. Lazan, Cell wall modifications, degrading enzymes and softening of carambola fruit during ripening, J. Exp. Bot. 50 (1999) 767–775.

[4] G.B. Seymour, Banana, in: G.B. Seymour, J.E. Taylor, G.A. Tucker (Eds.), Biochemistry of Fruit Ripening, Chapman Hall, London, UK, 1993, pp. 83–106.

[5] N. Narain, P.S. Bora, R. Narain, P.E. Shaw, Mango, in: P.E. Shaw, H.T. Chan, S. Nagy (Eds.), Tropical and Subtropical Fruits, Agscience Inc., Auburndale, FL, 1998, pp. 1–77.

[6] H. Lazan, Z.M. Ali, Guava, in: P.E. Shaw, H.T. Chan, S. Nagy (Eds.), Tropical and Subtropical Fruits, Agscience Inc., Auburndale, FL, 1998, pp. 446–485.

[7] T.J. O'Hare, Carambola, in: S.K. Mitra (Ed.), Postharvest Physiology and Storage of Tropical and Subtropical Fruits, CAB International, Wallingford, UK, 1997, pp. 295–307.

[8] L.H. Chin, Softening enzymes and cell wall modifications during ripening of carambola and other selected tropical fruits, Ph.D. Thesis, Universiti Kebangsaan Malaysia, Bangi, Malaysia, 2000.

[9] C.B. Hall, Firmness of tomato tissues according to cultivars and ripeness, J. Am. Soc. Hort. Sci. 112 (1987) 663–665.

[10] J.J. Giovannoni, D. DellaPenna, A.B. Bennett, R.L. Fischer, Polygalacturonase and tomato fruit ripening, Hort. Rev. 13 (1992) 67–109.

[11] C.J. Brady, Fruit ripening, Annu. Rev. Plant Physiol. 38 (1987) 155–173.

[12] R.L. Fischer, A.B. Bennett, Role of cell wall hydrolases in fruit ripening, Annu. Rev. Plant Physiol. Plant Mol. Biol. 42 (1991) 675–703.

[13] J.K.C. Rose, A.B. Bennett, Cooperative disassembly of the cellulose–xyloglucan network of plant cell walls: parallels between cell expansion and fruit ripening, Trends Plant Sci. 4 (1999) 176–183.

[14] Y. Kitagawa, Y. Kanayama, S. Yamaki, Isolation of β-galactosidase fractions from Japanese pear: activity against native cell wall polysaccharides, Physiol. Plant. 93 (1995) 545–550.

[15] Z.M. Ali, S.Y. Ng, R. Othman, L.Y. Goh, H. Lazan, Isolation, characterization and significance of papaya β-galactanases to cell wall modification and fruit softening during ripening, Physiol. Plant. 104 (1998) 105–115.

[16] K.A. Hadfield, J.K.C. Rose, D.S. Yaver, R.M. Berka, A.B. Bennett, Polygalacturonase gene expression in ripe melon fruit supports a role for polygalacturonase in ripening-associated pectin disassembly, Physiol. Plant. 117 (1998) 363–373.

[17] D.L. Smith, K.C. Gross, A family of at least seven β-galactosidase genes is expressed during tomato fruit development, Plant Physiol. 123 (2000) 1173–1183.

[18] C.P. Soh, α- and β-Galactosidases in papaya fruit softening during ripening, Ph.D. Thesis, Universiti Kebangsaan Malaysia, Bangi, Malaysia, 2002.

[19] D.J. Huber, Polyuronide degradation and hemicellulose modifications in ripening tomato fruit, J. Am. Soc. Hort. Sci. 108 (1983) 405–409.

[20] T.G. McCollum, D.J. Huber, D.J. Cantliffe, Modification of polyuronides and hemicelluloses during muskmelon fruit softening, Physiol. Plant. 76 (1989) 303–308.

[21] E.J. Mitcham, R.E. McDonald, Cell wall modification during ripening of 'Keitt' and 'Tommy Atkins' mango fruit, J. Am. Soc. Hort. Sci. 117 (1992) 919–924.

[22] R.J. Redgwell, L.D. Melton, D.J. Brasch, Cell wall dissolution in ripening kiwifruit (*Actinidia deliciosa*). Solubilization of the pectic polymers, Physiol. Plant. 98 (1992) 71–81.

[23] E.M. O'Donoghue, D.J. Huber, J.D. Timpa, G.W. Erdos, J.K. Brecht, Influence of avocado (*Persea americana*) Cx-cellulase on the structural features of avocado cellulose, Planta 194 (1994) 573–584.

[24] H. Lazan, M.K. Selamat, Z.M. Ali, β-Galactosidase, polygalacturonase and pectinesterase in differential softening and cell wall modification during papaya fruit ripening, Physiol. Plant. 95 (1995) 106–112.

[25] P. Muda, G.B. Seymour, N. Errington, G.A. Tucker, Compositional changes in cell wall polymers during mango fruit ripening, Carbohydr. Polym. 26 (1995) 255–260.

[26] J.K.C. Rose, K.A. Hadfield, J.M. Labavitch, A.B. Bennett, Temporal sequence of cell wall disassembly in rapidly ripening melon fruit, Physiol. Plant. 117 (1998) 345–361.

[27] D.J. Cosgrove, Wall structure and wall loosening. A look backwards and forwards, Physiol. Plant. 125 (2001) 131–134.

[28] Z.M. Ali, C.J. Brady, Purification and characterization of the polygalacturonases of tomato fruits, Aust. J. Plant Physiol. 9 (1982) 155–169.

[29] D.J. Cosgrove, Loosening of plant cell walls by expansins, Nature 407 (2000) 321–326.

[30] H. Lazan, Z.M. Ali, R. Jamal, G.R. Chaplin, Comparative ripening in mangos in relation to tissue position and heat treatment, ASEAN Food J. 2 (1986) 121–125.

[31] H. Lazan, Z.M. Ali, K.S. Liang, K.L. Yee, Polygalacturonase activity and variation in ripening of papaya fruit with tissue depth and heat treatment, Physiol. Plant. 77 (1989) 93–98.

[32] W.W. Luchsinger, R.A. Cornesky, Reducing power by the dinitrosalicylic acid method, Anal. Biochem. 4 (1962) 346–347.

[33] K.C. Gross, A rapid and sensitive spectrophotometric method for assaying polygalacturonase using 2-cyanoacetamide, Hort. Sci. 17 (1982) 933–934.

[34] N. Blumenkrantz, G. Asboe-Hansen, New method for quantitative determination of uronic acids, Anal. Biochem. 54 (1973) 484–489.

[35] M.K.A. Dubois, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric method for determination of sugars and related substances, Anal. Chem. 28 (1956) 350–356.

[36] A.T. Carey, K. Holt, S. Picard, R. Wilde, G.A. Tucker, C.R. Bird, W. Schuch, G. Seymour, Tomato exo-(1-4)-β-D-galactanase—Isolation, changes during ripening in normal and mutant tomato fruit, and characterization of a related cDNA clone, Physiol. Plant. 108 (1995) 1099–1107.

[37] N.L. Wade, E.E. Kavanagh, D.G. Hockley, C.J. Brady, Relationship between softening and the polyuronides in ripening banana fruit, J. Sci. Food Agric. 60 (1992) 61–68.

[38] Z.M. Ali, H. Lazan, Guava, in: S.K. Mitra (Ed.), Postharvest Physiology and Storage of Tropical and Subtropical Fruits, CAB International, Wallingford, UK, 1997, pp. 145–165.

[39] F. Micheli, Pectin methylesterase: cell wall enzymes with important roles in plant physiology, Trends Plant Sci. 6 (2001) 414–419.

[40] Z.M. Ali, S. Armugam, H. Lazan, β-Galactosidase and its significance in ripening mango fruit, Phytochemistry 38 (1995) 1109–1114.

[41] G.S. Ross, T. Wegrzyn, E.A. MacRae, R.J. Redgwell, Apple β-galactosidase: activity against cell wall polysaccharides and characterization of a related cDNA clone, Physiol. Plant. 106 (1994) 521–528.

[42] G. Maclachlan, C.J. Brady, Multiple forms of 1,4-β-glucanase in ripening tomato fruits include a xyloglucanase activatable by xyloglucan oligosaccharides, Aust. J. Plant Physiol. 19 (1992) 137–146.

[43] K. Keegstra, K.W. Talmadge, W.D. Bauer, P. Albersheim, The structure of plant cell walls. III. A model of the walls of

suspension-cultured sycamore cells based on the interconnections of the macromolecular components, Physiol. Plant. 51 (1973) 188–196.
[44] J. Fu, A.J. Mort, More evidence for covalent attachment of xyloglucan to the rhamnogalacturonan of cotton suspension culture cell walls, Physiol. Plant. 108 (1995) S-123.
[45] J.E. Thompson, S.C. Fry, Evidence for covalent linkage between xyloglucan and acidic pectins in suspension-cultured rose cells, Planta 211 (2000) 275–286.