ELSEVIER

Food Chemistry 81 (2003) 555–560

Food Chemistry

www.elsevier.com/locate/foodchem

Modification of pectin in Japanese persimmon fruit during the sun-drying process

Md. Ali Asgar, Ryo Yamauchi, Koji Kato*

The United Graduate School of Agricultural Science, Gifu University, Science of Biological Resources, 1-1 Yanagido, Gifu 501-1193, Japan

Received 11 July 2002; received in revised form 8 October 2002; accepted 8 October 2002

Abstract

Cold-water-soluble pectic polysaccharides (CWPs) from fresh and sun-dried Japanese persimmon (*Diospyros kaki*) were fractionated through DEAE-cellulose (phosphate form) column and in both cases, CWPs were separated into five fractions, CWP1, CWP2, CWP3, CWP4 and CWP5. Each CWP3 was subjected to gel filtration chromatography on a Sepharose CL-6B column. Gel filtration showed that solubilization of pectin as high-molecular-weight polymer into CWP3 and depolymerization of CWP3 occurred during the sun-drying process. CWP3 (fresh persimmon) contained galactose as the predominant neutral sugar, whereas arabinose was the predominant neutral sugar in newly solubilized CWP3. A low-molecular-weight fraction of CWP3 (sun-dried persimmon) represented mainly the rhamnogalacturonan backbone of 'hairy' regions. The molecular size distribution of CWP3 was affected due to degradation of side-chains. Loss of arabinose occurred at a slower rate than galactose from CWP3. The results imply a softening-associated modification of Japanese persimmon pectin during the sun-drying process.
© 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Japanese persimmon; Sun-drying process; Pectin modification; Pectin fractionation; Side-chain of pectin

1. Introduction

Japanese persimmon cultivars (*Diospyros kaki*) are usually classified into two groups, an astringent and a non-astringent type, depending on the degree of astringency at the mature stage (Matsuo, 1998). When an astringent persimmon fruit is eaten, the tannin cells in the flesh are crushed and soluble tannins are released, giving a strong astringent sensation (Taira, 1996). Such cultivars are inedible even when fully developed.

Astringency can be removed from astringent persimmon fruits by various treatments, including ethanol vapour treatment of fruit on the tree or after harvest, drying of peeled fruit, carbon dioxide gas treatment, immersion in warm water and freezing of fruit (Taira, 1996). Astringency disappears when soluble tannins become insoluble (Matsuo, 1998). Generally, the sun-drying process is used to remove astringency from Japanese persimmon fruits. In addition to the changes in taste and palatability, softening of Japanese persimmon occurs during the sun-drying process.

Fruit softening, which characterizes ripening, involves structural and compositional changes of the cell wall carbohydrates, mainly as a result of the action of wall-degrading enzymes (Ali, Ng, Othman, Goh, & Lazan, 1998). It is found that tannins inactivate cell wall-degrading enzymes (Dennis, 1987). These enzyme activities can be enhanced in Japanese persimmon fruits during the sun-drying process due to insolubilization of tannins.

Among the cell wall-degrading enzymes, pectin-degrading enzymes have received most attention as potential causal agents in the softening of ripening fruit (Cutillas-Iturralde, Zarra, & Lorences, 1993).

Architecturally, pectins and other components of the primary wall matrix are stabilized by the presence of inter- as well as intra-molecular cross-links, making the wall very coherent and water insoluble (Fry, 1986). The hairy domains of pectins, the rhamnogalacturonan 1, contain neutral sugar side-chains that are enriched with arabinosyl and galactosyl residues, but to a varying extent, depending on the fruit type (Schols, Voragen, & Colquhoun, 1994).

* Corresponding author. Fax: +81-58-293-2925.
E-mail address: katokoji@cc.gifu-u.ac.jp (K. Kato).

0308-8146/03/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0308-8146(02)00489-2

We now report softening-associated changes in the 'hairy' regions of Japanese persimmon pectin during the sun-drying process.

2. Materials and methods

2.1. Materials

Fresh and sun-dried Japanese persimmon (*D. kaki*, cv. Ijiraomi) fruits were obtained from a commercial grower in Gifu, Japan. A batch of fresh Japanese persimmon fruits were brought to the laboratory immediately upon harvest and, in a short time, the fruits was peeled, and excised into small pieces for freeze-drying. Another batch of fresh fruits was peeled, and sun-dried by the commercial grower. The duration of the sun-drying process was 1 month. Without delay, sun-dried Japanese persimmon fruits were transported to the laboratory, excised into small pieces, and the remaining water was removed by freeze-drying.

2.2. Methods

2.2.1. Isolation of cold-water-soluble pectic polysaccharides (CWPs)

CWPs were isolated from fresh Japanese persimmon and sun-dried Japanese persimmon fruits. Each dry sample (100 g) was defatted with a mixture of chloroform and methanol (2:1 v/v). Each defatted sample was extracted 3 times with distilled water (1.8 L) at 4 °C for 4 h. After centrifugation, the supernatant was concentrated, then treated with 5 vol. of 99% ethanol, and kept overnight at 4 °C. Each precipitate was mixed with distilled water, then dialysed against distilled water, and freeze-dried.

2.2.2. General methods

The total sugar was determined by the phenol-H_2SO_4 method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), and uronic acid by the carbazole-H_2SO_4 method (Dische, 1962). The neutral sugar content was determined by subtraction of the value of uronic acid content from total sugar content. Paper chromatography of uronic acids was performed on Toyo No. 50 filter paper with the solvent system, 1-butanol: pyridine: water (6:4:3, v/v). Glycosyl residues (neutral sugars) of the polysaccharides were analyzed by gas-liquid chromatography (GLC) as alditol acetates after acid hydrolysis (Blakeney, Harris, Henry, & Stone, 1983). GLC was carried out on a Shimadzu GC-18A apparatus equipped with a flame ionization detector. A capillary column (25 m×0.22 mm i.d., 0.25 μm Hicap CBP-10) was used and operated at 220 °C with a gas flow rate of 60 ml/min of nitrogen. Peak areas were measured with a Shimadzu Chromatocorder 21. The ratio of neutral sugars was calculated from the GLC peak areas.

2.2.3. DEAE-cellulose column chromatography

DEAE-cellulose column chromatography was performed according to the method of Shibuya and Nakane (1984). Each CWPs sample (25–50 mg) was loaded onto a column (2×15 cm) of DEAE-cellulose (phosphate form). In both cases, the fractions were eluted sequentially with 250 ml of 0.05 M phosphate buffer (pH 6.0) and successively by 250 ml of buffer containing, respectively, 0.05, 0.125, 0.25, and 0.5 M NaCl at 40 ml/h flow rate. The carbohydrate was not eluted with 250 ml of buffer containing 1 M NaCl. The fractions, CWP1 (buffer), CWP2 (0.05 M NaCl), CWP3 (0.125 M NaCl), CWP4 (0.25 M NaCl), and CWP5 (0.5 M NaCl) were dialysed against distilled water, and freeze-dried.

2.2.4. Gel-filtration chromatography

Each CWP3 sample (2 to 5 mg) was loaded onto a column (1.6×93 cm) of Sepharose CL-6B and eluted with 0.15 M sodium acetate buffer (pH 4.0) at a flow rate of 18 ml/h. The void volume (V_0), and the total volume (V_t) of the column were determined as the elution volume of Dextran T 2000 and galacturonic acid, respectively. Dextran T 500 (molecular weight 487,000), Dextran T 110 (molecular weight 105,000), and Dextran T 10 (molecular weight 10,500) were used to calibrate molecular weight.

3. Results and discussion

The yields, and sugar content, of cold-water-soluble pectic polysaccharides (CWPs) from fresh and sun-dried Japanese persimmon fruits are listed in Table 1. The presence of galacturonic acid in the hydrolyzate of CWPs from fresh and sun-dried fruits was confirmed by paper chromatography. Table 1 shows that the yield of CWPs was increased about 44% during the sun-drying process. This result indicates that extensive solubilization of pectins occurred during the sun-drying process. As a fruit ripens, a substantial portion of its cell wall pectins are converted to a water-soluble form and these changes are of considerable importance in fruit texture (Ben-Arie, Kislev, & Frenkel, 1979; Labavitch, 1981).

Table 1
Yield, and sugar content of CWPs from fresh and sun-dried Japanese persimmon

Fraction	Yield[a] (g/100 g)	Uronic acid (%)	Neutral sugar (%)
Fresh	1.6	24.5	64.7
Sun-dried	2.3	38.1	52.2

[a] Expressed as dry-weight basis.

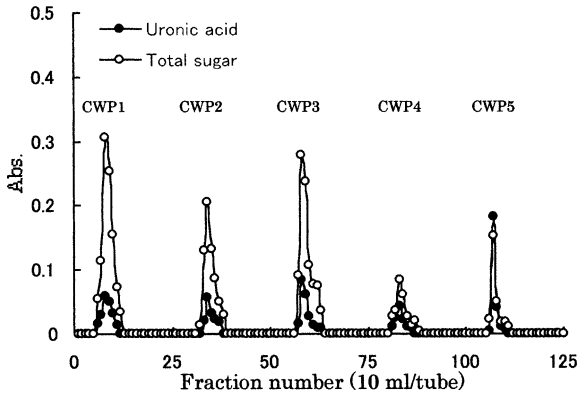

Fig. 1. DEAE-cellulose column chromatography (phosphate form) of CWPs from fresh Japanese persimmon. Experimental conditions are described in Section 2.

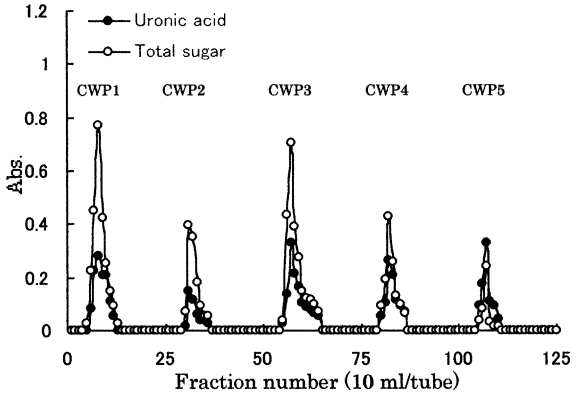

Fig. 2. DEAE-cellulose column chromatography (phosphate form) of CWPs from sun-dried Japanese persimmon. Experimental conditions are described in Section 2.

Figs. 1 and 2 show the elution profiles of the CWPs from fresh and sun-dried fruits on DEAE-cellulose (phosphate form) column, respectively. Tables 2 and 3 show the yield, sugar content, and neutral sugar composition of DEAE-cellulose purified pectic polysaccharides from fresh and sun-dried fruits, respectively. As expected, during the DEAE-cellulose fractionation, the uronic acid/neutral sugar ratio increased with the ionic strength. In fresh and sun-dried persimmon, a non-retained fraction (CWP1) contained over 85% neutral sugar and low levels (8.4–11.2%) of uronic acid.

In both cases, the pectic polysaccharides retained on the DEAE-cellulose column were separated sequentially into four fractions, CWP2, CWP3, CWP4, and CWP5. These fractions were neutral sugar-rich pectic polysaccharides except for CWP5. Galactose, arabinose, rhamnose, glucose, and mannose were present as neutral sugars in these fractions of fresh and sun-dried fruits, though in different proportions.

Sugar composition of all fractions from fresh persimmon was found to alter during the sun-drying process, indicating that modification of pectic polysaccharides occurred during this time. The most striking change was a deficiency of galactose in these fractions from sun-dried persimmon. During ripening, there are marked losses of galactose from kiwifruit, tomato, watermelon, apple (Braeburn), apple (Cox), nashi pear and avocado (Redgwell, Fischer, Kendal, & MacRae, 1997).

Both CWP5s seem to be contaminated with xyloglucan and this may signify a close association between pectins and hemicelluloses in the cell wall. Xyloglucan was present as the main polysaccharide in the hemicelluloses of persimmon fruit (Cutillas-Iturralde, Zarra, Fry, & Lorences, 1994). In our study, loss of xylose and glucose occurred from CWP5 during the sun-drying process, possibly due to the degradation of xyloglucan. Softening-associated decomposition of hemicellulose was found in astringent persimmon of the cultivar Hiratanenashi during alcohol vapour treatment and carbon dioxide gas treatment (Itamura, Fukushima, & Kitamura, 1989).

The major retained fractions, CWP3, of fresh and sun-dried persimmon were separately subjected to gel permeation chromatography on a Sepharose CL-6B column to determine the molecular weight distribution by using authentic dextrans from Pharmacia Fine Chemicals. CWP3 (fresh) gave a symmetrical peak (elution vol. 103–120 ml) through Sepharose CL-6B column

Table 2
Yield, sugar content, and neutral sugar composition of DEAE-cellulose purified pectic polysaccharides from fresh Japanese persimmon

Fraction	Yield[a] (%)	Uronic acid (%)[b]	Neutral sugar (%)[b]	Neutral sugar composition (mol%)[b]					
				Rha	Ara	Xyl	Man	Gal	Glc
CWP1	26.6	8.4	87.7	1.2	39.8	ND[c]	1.4	53.9	1.6
CWP2	16.7	19.2	78.3	4.5	37.2	ND	0.9	49.3	5.0
CWP3	23.2	22.1	75.1	5.6	42.0	ND	Tr[c]	46.1	4.5
CWP4	6.4	34.8	63.0	13.1	41.1	3.0	4.6	23.9	12.9
CWP5	14.4	72.2	23.9	14.2	29.7	19.5	4.8	16.2	13.8

[a] Expressed as dry-weight basis.
[b] Each value is the mean of three replicates and for any value the error was less than 5%.
[c] ND, none detected; Tr, trace.

Table 3
Yield, sugar content, and neutral sugar composition of DEAE cellulose purified pectic polysaccharides from sun-dried Japanese persimmon

Fraction	Yield[a] (%)	Uronic acid (%)[b]	Neutral sugar (%)[b]	Neutral sugar composition (mol%)[b]					
				Rha	Ara	Xyl	Man	Gal	Glc
CWP1	25.7	11.2	85.2	2.4	46.0	2.5	5.9	25.1	13.8
CWP2	12.0	21.4	75.5	10.0	50.0	ND[c]	1.2	34.9	2.3
CWP3	22.9	31.2	66.4	16.2	58.6	ND	Tr[c]	21.2	1.4
CWP4	16.6	38.6	57.7	29.8	52.8	ND	1.5	12.3	2.5
CWP5	10.7	81.4	15.2	21.9	23.4	14.5	9.9	14.2	12.6

[a] Expressed as dry-weight basis.
[b] Each value is the mean of three replicates and for any value the error was less than 5%.
[c] ND, none detected; Tr, trace.

and the molecular-weight distribution was 105,000–329,000 (Fig. 3). On the other hand, the Sepharose CL-6B column fractions from CWP3 (sun-dried) were divided into three aliquots, 'a' (elution vol. 103–120 ml), 'b' (elution vol. 121–156 ml) and 'c' (elution vol. 157–171 ml), showed in Fig. 4. The molecular-weight distribution was 105,000–329,000 (for 'a') and 10,500–103,500 (for 'b'). The molecular weight of Fraction 'c' was less than 10,500.

CWP3 (fresh) was recovered as a high molecular weight polymer through the Sepharose CL-6B column chromatography, whereas in addition to the high molecular weight polymer, intermediate and low molecular weight polymers were obtained from CWP3 (sun-dried). It is clear that depolymerization of CWP3 occurred during the sun-drying process. Nevertheless, the high molecular weight polymer did not disappear during this time. CWP3 (fresh) and Fraction 'a' had the same molecular weight distributions, indicating that pectic solubilization also occurred during the sun-drying process. Increased solubilization and depolymerization of pectic polysaccharides have been observed during the ripening of many fruit types (Wakabayashi, Chun, & Huber, 2000) and seem to be important determinants of the textural changes in Japanese persimmon fruit during the sun-drying process. Newly solubilized CWP3 was of high molecular weight, indicating that extensive pectin metabolism is not necessary for solubilization. Similar results were found in nectarine fruit (Dawson, Melton, & Watkins, 1992) and 'Bartlett' pear fruit (Ahmed & Labavitch, 1980).

After gel filtration chromatography, the sugar content and neutral sugar composition of CWP3 (fresh) were determined and were the same as reported for CWP3 in Table 2. The sugar content and neutral sugar composition of fractions 'a', 'b', and 'c' are shown Table 4. CWP3 (fresh), 'a', 'b' and 'c' fractions contained, respectively, 22.1, 26.4, 30.1 and 40.4% of uronic acid. The neutral sugar data reported in Table 2 and Table 4 showed that CWP3 (fresh), 'a', 'b', and 'c' Fractions were composed mainly of rhamnose, arabinose and galactose, but in different ratios. The ratio of rhamnose: arabinose: galactose was 1: 7.5: 8.2 for CWP3 (fresh), 1: 9.2: 5.2 for 'a', 1: 6.4: 1.4 for 'b', and 1: 0.6: 0.3 for 'c'.

The galactose/rhamnose and arabinose/rhamnose ratio were found to decrease with downshifts in molecular mass of CWP3, suggesting that loss of galactose

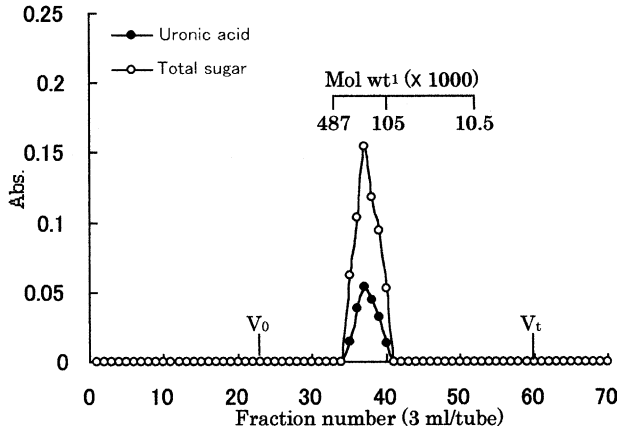

Fig. 3. The molecular weight distribution of CWP3 from fresh Japanese persimmon on Sepharose CL-6B column. Experimental conditions are described in Section 2. [1] Mol wt, molecular weight.

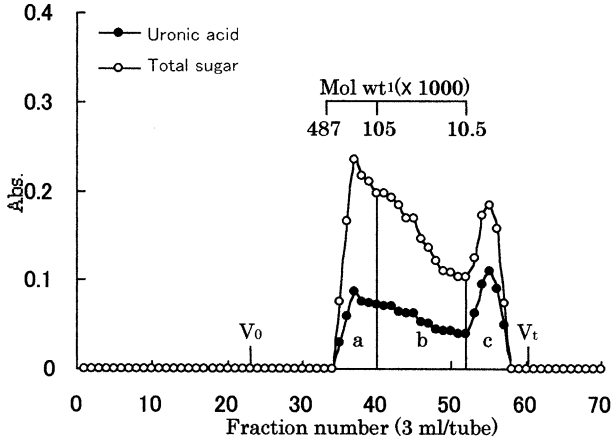

Fig. 4. The molecular weight distribution of CWP3 from sun-dried Japanese persimmon on Sepharose CL-6B column. Experimental conditions are described in Section 2. [1] Mol wt, molecular weight.

Table 4
Sugar content, and neutral sugar composition of fractions 'a', 'b', and 'c'

Fraction	Uronic acid (%)[a]	Neutral sugar (%)[a]	Neutral sugar composition (mol%)[a]				
			Rha	Ara	Man	Gal	Glc
'a' (103–120 ml)	26.4	71.2	6.3	57.8	Tr	32.6	1.2
'b' (121–156 ml)	30.1	68.0	10.9	69.9	Tr[b]	15.5	1.7
'c' (157–171 ml)	40.4	57.5	48.9	30.5	4.0	13.7	2.9

[a] Each value is the mean of three replicates and for any value the error was less than 5%.
[b] Tr, trace.

and arabinose occurred as an ethanol-soluble form. There was an enrichment of rhamnose in the low molecular weight fraction, at the expense of galactose and arabinose. High levels of uronic acid and rhamnose in fraction 'c' represent mainly the rhamnogalacturonan backbone, indicating the presence of 'hairy' regions in CWP3 of Japanese persimmon fruit. In the 'hairy' regions, the backbone is composed of 4-linked α-D-galacturonic acid and 2-linked α-L-rhamnose (Renard, Crepeau, & Thibault, 1995). Neutral sugars, as side-chains, are covalently linked to the rhamnogalacturonan via the C-4 of the rhamnosyl residues (Eda & Kato, 1980; McNeil, Darvill, & Albersheim, 1980; Talmadge, Keegstra, Bauer, & Albersheim, 1973). Side-chain sugars are predominantly galactose and arabinose forming galactan, arabinan and/or arabinogalactan polymers (Thibault, 1983). It has been established by enzymic degradation that most of the side chains are located on relatively small proportions of the backbone (Rombouts & Thibault, 1986). Evidently, during the sun-drying process, the molecular size distribution of CWP3 was affected due to degradation of side-chains.

The rhamnose:arabinose:galactose ratio analysis of Sepharose CL-6B column fractions revealed that loss of arabinose occurred at a slower rate than galactose. Perhaps, this was due to different levels of β-galactosidase and α-arabinosidase activities. During ripening, higher levels of β-galactosidase activity compared to α-arabinosidase activity were found in many fruit types, such as mango (Ali, Armugam, & Lazan, 1995) and papaya (Ali et al., 1998). In vitro, β-galactosidase was able to catalyze pectin depolymerization (Ali et al., 1998; De Veau, Gross, Huber, & Watada, 1993). α-Arabinosidase also has the capacity to modify pectins as a pectin debranching enzyme (Guillon, Thibault, Rombouts, Voragen, & Pilnik, 1989).

The rhamnose-to-galactose ratio in Fraction 'c' implied that the galactose residues were present in this fraction as very short chains attached to the rhamnogalacturonan backbone. Therefore, it seems that the enzyme failed to remove all the galactose from the backbone. The treatment of sugar-beet pectins with β-D-galactosidase and endo-(1→4)-β-D-galactanase gave similar results (Guillon et al., 1989). Perhaps the enzyme cleaved all the galactose-to-galactose linkages of Fraction 'c' but failed to cleave the backbone-to-galactose linkages during sun-drying process. Fraction 'c' contained less arabinose than that in any of CWP3 (fresh), 'a' and 'b' fractions.

Tables 2 and 4 show that CWP3 from fresh fruit contained galactose as the predominant neutral sugar, whereas arabinose was the predominant neutral sugar in the newly solubilized CWP3 (fraction 'a'). It is reasonable to assume that softening-associated loss of arabinose occurred at a slower rate than galactose from 'proto-pectin' (the pectin in the cell wall) during the sun-drying process, as found in CWP3. A marked deficiency of galactose in fraction 'a' may imply that there was a correlation between degradation of side-chains and pectic solubilization during the sun-drying process. In vitro, papaya β-galactosidase was able to catalyze, not only the depolymerization but also the solubilization of the wall pectins from unripe papaya fruits (Ali et al., 1998). This ability of β-galactosidase to modify pectins occurred in the absence of polygalacturonase activity. More experiments will be needed to clarify the mode of pectic solubilization in Japanese persimmon fruits during the sun-drying process.

References

Ahmed, A. E., & Labavitch, J. M. (1980). Cell wall metabolism in ripening fruit. *Plant Physiology*, *65*, 1009–1013.

Ali, Z. M., Armugam, S., & Lazan, H. (1995). β-Galactosidase and its significance in ripening mango fruit. *Phytochemistry*, *38*(5), 1109–1114.

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

Ben-Arie, R., Kislev, N., & Frenkel, C. (1979). Ultrastructural changes in the cell walls of ripening apple and pear fruit. *Plant Physiology*, *64*, 197–202.

Blakeney, A. B., Harris, P. J., Henry, R. J., & Stone, B. A. (1983). A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydrate Research*, *113*, 291–299.

Cutillas-Iturralde, A., Zarra, I., Fry, S. C., & Lorences, E. P. (1994). Implication of persimmon fruit hemicellulose metabolism in the softening process. Importance of xyloglucan endotransglycosylase. *Physiologia Plantarum*, *91*, 169–176.

Cutillas-Iturralde, A., Zarra, I., & Lorences, E. P. (1993). Metabolism of cell wall polysaccharides from persimmon fruit. Pectin solubilization during fruit ripening occurs in apparent absence of polygalacturonase activity. *Physiologia Plantarum*, *89*, 369–375.

Dawson, D. M., Melton, L. D., & Watkins, C. B. (1992). Cell wall changes in nectarines (*Prunus persica*). *Plant Physiology*, *100*, 1203–1210.

Dennis, C. (1987). Fungi. In J. Weichmann (Ed.), *Postharvest physiology of vegetables* (pp. 377–411). New York: Marcel Dekker.

De Veau, I. E. J., Gross, K. C., Huber, D. J., & Watada, A. E. (1993). Degradation and solubilisation of pectin by β-galactosidases purified from avocado mesocarp. *Physiologia Plantarum*, *87*, 279–285.

Dische, Z. (1962). Color reactions of hexuronic acids. In R. L. Whistler, & M. L. Wolfrom (Eds.), *Methods in carbohydrate chemistry* (Vol. 1, pp. 497–501). New York: Academic Press.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*(3), 350–356.

Eda, S., & Kato, K. (1980). Pectin isolated from the midrib of leaves of *Nicotiana tabacum*. *Agricultural & Biological Chemistry*, *44*(12), 2793–2801.

Fry, S. C. (1986). Cross-linking of matrix polymers in the growing cell walls of angiosperms. *Annual Review Plant Physiology*, *37*, 165–186.

Guillon, F., Thibault, J.-F., Rombouts, F. M., Voragen, A. G. J., & Pilnik, W. (1989). Enzymic hydrolysis of the "hairy" fragments of sugar-beet pectins. *Carbohydrate Research*, *190*, 97–108.

Itamura, H., Fukushima, T., & Kitamura, T. (1989). Changes in the cell wall polysaccharide composition during fruit softening of Japanese persimmons (*Diospyros kaki* Thunb. var Hiratanenashi). *Nippon Shokuhin Kogyo Gakkaishi*, *36*(8), 647–650.

Labavitch, J. M. (1981). Cell wall turnover in plant development. *Annual Review Plant Physiology*, *32*, 385–406.

Matsuo, T. (1998). Chemistry, metabolism and biological functions of fruit tannins and relative compounds. *Recent Research Developments in Phytochemistry*, *2*, 269–285.

McNeil, M., Darvill, A. G., & Albersheim, P. (1980). Structure of plant cell walls. *Plant Physiology*, *66*, 1128–1134.

Redgwell, R. J., Fischer, M., Kendal, E., & MacRae, E. A. (1997). Galactose loss and fruit ripening: high-molecular-weight arabinogalactans in the pectic polysaccharides of fruit cell walls. *Planta*, *203*, 174–181.

Renard, C. M. G. C., Crepeau, M.-J., & Thibault, J.-F. (1995). Structure of the repeating units in the rhamnogalacturonic backbone of apple, beet and citrus pectins. *Carbohydrate Research*, *275*, 155–165.

Rombouts, F. M., & Thibault, J.-F. (1986). Enzymic and chemical degradation and the fine structure of pectins from sugar-beet pulp. *Carbohydrate Research*, *154*, 189–203.

Schols, H. A., Voragen, A. G. J., & Colquhoun, I. J. (1994). Isolation and characterization of rhamnogalacturonan oligomers, liberated during degradation of pectic hairy regions by rhamnogalacturonase. *Carbohydrate Research*, *256*, 97–111.

Shibuya, N., & Nakane, R. (1984). Pectic polysaccharides of rice endosperm cell walls. *Phytochemistry*, *23*(7), 1425–1429.

Taira, S. (1996). Astringency in persimmon. In H.-F. Linskens, & J. F. Jackson (Eds.), *Modern methods of plant analysis, fruit analysis* (pp. 97–110). Berlin, Heidelberg: Springer-Verlag.

Talmadge, K. W., Keegstra, K., Bauer, W. D., & Albersheim, P. (1973). The structure of plant cell walls. *Plant Physiology*, *51*, 158–173.

Thibault, J.-F. (1983). Enzymatic degradation and β-elimination of the pectic substances in cherry fruits. *Phytochemistry*, *22*(7), 1567–1571.

Wakabayashi, K., Chun, J.-P., & Huber, D. J. (2000). Extensive solubilization and depolymerization of cell wall polysaccharides during avocado (*Persea americana*) ripening involves concerted action of polygalacturonase and pectinmethylesterase. *Physiologia Plantarum*, *108*, 345–352.