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Structural features of pectins from fresh and sun-dried Japanese persimmon fruit

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

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Abstract

Softening-associated changes in a cold-water-soluble pectin, CWP3 of Japanese persimmon fruit are investigated by methylation analysis. Methylation analysis showed that rhamnose occurred as $(1 \rightarrow 2)$- and $(1 \rightarrow 2,4)$-linked residues in CWP3 (from fresh persimmon), newly solubilized CWP3 (fraction 'a' from sun-dried persimmon) and depolymerized CWP3 (fraction 'b' from sun-dried persimmon) as expected for the presence of 'hairy' regions. Solubilization of pectin into CWP3 occurred with higher proportions of $(1 \rightarrow 5)$-linked arabinan side-chains and lower proportions of $(1 \rightarrow 3)/(1 \rightarrow 6)$-linked arabinogalactan side-chains compared to the respective side chains in CWP3 (fresh persimmon). During the sun-drying process, marked losses of $(1 \rightarrow 3,6)$-linked galactose residues from CWP3 occurred, due to extensive degradation of main-chains of arabinogalactans. This implied losses of both types of sugars, arabinose (as terminal) and galactose from arabinogalactans in CWP3 of Japanese persimmon. During the sun-drying process, the molecular size distribution of CWP3 was affected due to degradation of arabinogalactan and arabinan side-chains.
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Keywords: Japanese persimmon; Sun-drying process; Softening; Pectin; Arabinogalactan; Arabinan

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 (1 month duration) 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.

In our previous study (Asgar, Yamauchi, & Kato, 2003), the molecular-weight distribution and the sugar composition analysis showed softening-associated changes in a cold-water-soluble pectin, CWP3 of Japanese persimmon fruit during the sun-drying process.

In the previous study (Asgar et al., 2003), cold-water-soluble pectic polysaccharides (CWPs) from fresh and sun-dried Japanese persimmon were fractionated with a DEAE-cellulose (phosphate form) column and in both cases, CWPs were separated into five fractions, CWP1, CWP2, CWP3, CWP4 and CWP5; the major retained fractions, CWP3, of fresh and sun-dried persimmon were separately subjected to gel permeation chromatography on a Sepharose CL-6B column (1.6×93 cm) to determine the molecular weight distribution by using authentic dextrans. CWP3 (fresh) was recovered as a high molecular weight polymer (a symmetrical peak, elution vol. 103–120 ml) through the Sepharose CL-6B column chromatography whereas, in addition to the

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

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high molecular weight polymer, 'a' (elution vol. 103–120 ml), an intermediate molecular weight polymer, 'b' (elution vol. 121–156 ml) and low molecular weight polymer, 'c' (elution vol. 157–171 ml) were obtained from CWP3 (sun-dried). CWP3 (fresh), 'a', 'b' and 'c' fractions contained, respectively, 22.1%, 26.4%, 30.1% and 40.4% of uronic acid; galactose, arabinose and rhamnose were mainly present as neutral sugars in these fractions, but in different proportions.

The previous analysis (Asgar et al., 2003) indicated that solubilization of pectin, as the high-molecular-weight polymer, into CWP3 and depolymerization of CWP3 (into intermediate and then low molecular weight polymer) occurred during the sun-drying process. The molecular size distribution of CWP3 was affected due to loss of galactose and arabinose (neutral sugars side-chains) and a low-molecular-weight fraction of CWP3 (sun-dried persimmon) represented mainly the rhamnogalacturonan backbone of 'hairy' regions (Asgar et al., 2003).

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 (Asgar et al., 2003). In the present study, CWP3 (from fresh persimmon), newly solubilized CWP3 (fraction 'a' from sun-dried persimmon) and depolymerized CWP3 (fraction 'b' from sun-dried persimmon) were methylated in order to obtain more information about softening-associated changes in 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 treated as mentioned in our preceding paper (Asgar et al., 2003).

2.2. Methods

2.2.1. Pectin

Both CWP3s were obtained, and then fractionated as described in the preceding paper (Asgar et al., 2003).

2.2.2. Infrared (IR) spectroscopy

IR spectra were recorded with a Perkin Elmer 2000 FT–IR spectrometer system.

2.2.3. Gas-liquid chromatography (GLC)

GLC was carried out in a Shimadzu GC-18A apparatus equipped with a flame ionization detector. A capillary column of CBP-1-M25-025 (0.22 mm × 25 m) was used. Peak areas were measured with a Shimadzu Chromatocorder 21.

2.2.4. Gas chromatography-mass spectrometry (GC–MS)

GC–MS was conducted with a Shimadzu GC–MS QP-5000 apparatus, with a class 5000 mass data system. A methyl silicon DB-1 capillary column (30 m × 0.25 mm × 1 μm, J&W Scientific) was used. The column temperature was programmed first at 150 °C for 2 min, and then raised to 250 °C at 5 °C/min. The spectra were recorded at an ionizing potential of 70 eV.

2.2.5. Neutral sugar linkage composition

CWP3 (fresh), newly solubilized CWP3 (fraction 'a') and depolymerized CWP3 (fraction 'b') were methylated by the Hakomori method, as modified by Harris, Henry, Blakeney, and Stone (1984). Each product was confirmed to show no absorption for the hydroxyl group in its IR spectrum. The methylated polysaccharides were hydrolyzed with 90% formic acid, and then with 0.25 M sulfuric acid. The partially methylated sugars thus obtained were converted to their alditol acetates (Blakeney, Harris, Henry, & Stone, 1983) and the partially methylated alditol acetates were analyzed by GLC and GC–MS.

3. Results and discussion

The results of methylation analysis of CWP3 (fresh), newly solubilized CWP3 (fraction 'a') and depolymerized CWP3 (fraction 'b') are shown in Table 1.

Table 1
Neutral sugar linkage composition of CWP3 (fresh), 'a' (sun-dried) and 'b' (sun-dried) fractions

Sugar linkage	Mol (%)		
	CWP3 (fresh)	'a' (sun-dried)	'b' (sun-dried)
Arabinose			
Araf-(1→	20.6	20.0	13.1
→5)-Araf-(1→	19.0	34.4	56.0
→3,5)-Araf-(1→	4.3	9.0	10.5
Total	43.9	63.4	79.6
Galactose			
Galp-(1→	3.9	2.2	1.3
→3)-Galp-(1→	8.3	6.9	3.2
→6)-Galp-(1→	5.5	2.8	1.1
→3,6)-Galp-(1→	27.3	17.5	5.6
Total	45.0	29.4	11.2
Rhamnose			
→2)-Rhap-(1→	1.8	2.2	3.5
→2,4)-Rhap-(1→	2.4	2.6	3.6
Total	4.2	4.8	7.1
Unknown components[a]	6.9	2.4	2.1

[a] In each fraction, the identities of some minor components were not confirmed.

In the preceding paper (Asgar et al., 2003), the sugar composition showed that a low-molecular-weight fraction of CWP3 (fraction 'c' from sun-dried persimmon) represented mainly the rhamnogalacturonan backbone, indicating the presence of 'hairy' regions in CWP3 of Japanese persimmon fruit. In the present study, methylation analysis revealed that rhamnose occurred as $(1 \rightarrow 2)$- and $(1 \rightarrow 2,4)$-linked residues in CWP3 (fresh), 'a' and 'b' fractions, as expected for the presence of 'hairy' regions. In this region, the backbone is composed of 4-linked α-D-galacturonic acid and 2-linked α-L-rhamnose (Renard, Crepeau, & Thibault, 1995; Vignon & Garcia-Jaldon, 1996). Neutral sugars, as side-chains, are covalently linked to the rhamnogalacturonan via the C-4 of the rhamnosyl residues (Eda & Kato, 1980; Shibuya & Nakane, 1984; Vignon & Garcia-Jaldon, 1996). Sometimes, in addition to the linkage via position 4 of the rhamnosyl residues, attachments of side chains to the rhamnogalacturonan backbone via position 3 of the galacturonosyl residues are also found (Ishii, 1981).

Arabinose (Araf) was found as terminal, $(1 \rightarrow 5)$- and $(1 \rightarrow 3,5)$-linked residues in CWP3 (fresh), 'a' and 'b' fractions, suggesting the presence of $(1 \rightarrow 5)$-linked arabinans with additional branches at the O-3 of the Araf residues in these fractions. 1,2-Araf and 1,2,5-Araf residues were not detected in these fractions. A low proportion of branched arabinose (15.8%–20.7% of 1,5-linked arabinose) residues was found in CWP3 (fresh), 'a' and 'b' fractions, indicating a relatively low degree of branching of the arabinans present in these fractions compared to arabinans in other pectins (Guillon & Thibault, 1989; McNeil, Darvill, & Albersheim, 1980).

In CWP3 (fresh), 'a' and 'b' fractions, galactose (Galp) residues were terminal, $(1 \rightarrow 3)$-, $(1 \rightarrow 6)$- and $(1 \rightarrow 3,6)$-linked, indicating the presence of Type II (arabino) galactans in these fractions. $(1 \rightarrow 3)/(1 \rightarrow 6)$-Linked galactans have been found as side chains in pectins from different sources (Duan, Wang, Dong, Fang, & Li, 2003; de Vries, den Uijl, Voragen, Rombouts, & Pilnik, 1983).

High proportions of $(1 \rightarrow 3,6)$-linked galactose residues (T-Galp = 3.9%, 1,3-Galp = 8.3%, 1,6-Galp = 5.5% and 1,3,6-Galp = 27.3%) and also high proportions of terminal arabinose residues (46.9% of total arabinose) were found in CWP3 of fresh persimmon (Table 1). Table 1 also shows that low proportions of branched arabinose residues (18.5% of 1,5-linked arabinose residues) were present in CWP3 (fresh). Because of the high degree of branching (high proportions of 1,3,6-Galp residues) of the $(1 \rightarrow 3)/(1 \rightarrow 6)$-linked arabinogalactans in CWP3 (fresh) and the low degree of branching of the arabinans, it is very likely that a relative large proportion of the terminal arabinose residues originated from the side chains of $(1 \rightarrow 3)/(1 \rightarrow 6)$-linked arabinogalactans. Talmadge, Keegstra, Bauer, and Albersheim (1973) also reported that this type of arabinogalactan is characterized by a branched framework of 3- and 6-linked galactosyl residues and most of the side chains in this type of arabinogalactan are single arabinofuranosyl residues.

In our preceding paper (Asgar et al., 2003), the sugar composition showed 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') and it was assumed 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. The results of methylation analysis of CWP3 (fresh) and fraction 'a' in the present study are consistent with sugar composition of these fractions. Methylation analysis showed that CWP3 (fresh) contained 19% $(1 \rightarrow 5)$-linked arabinose residues, whereas 34.4% $(1 \rightarrow 5)$-linked arabinose residues were present in fraction 'a', indicating that solubilization of pectin into CWP3 occurred with relatively high proportions of arabinans side-chains compared to that in CWP3 (fresh). Methylation analysis also showed the deficiency of $(1 \rightarrow 3)$-, $(1 \rightarrow 6)$- and $(1 \rightarrow 3,6)$-linked galactose residues in fraction 'a', suggesting that relatively lower proportions of galactan side-chains were present in the newly solubilized CWP3 than in CWP3 (fresh). This deficiency in fraction 'a' may imply that there was a correlation between degradation of galactan side-chains and pectic solubilization during the sun-drying process. Degradation of galactan side-chains may allow loosening of the structure of the wall and improved solubilization of pectins.

The glycosidic linkage composition showed that slightly high proportions of branched arabinose residues (20.7% of 1,5-linked arabinose residues) were present in the newly solubilized CWP3 (fraction 'a') compared to that (18.5% of 1,5-linked arabinose residues) in CWP3 of fresh persimmon. Nevertheless, lower proportions of terminal arabinose residues (31.5% of total arabinose) were found in the newly solubilized CWP3 than that (46.9% of total arabinose) in CWP3 (fresh). The glycosidic linkage composition also showed that CWP3 (fresh) had a higher level (27.3%) of 1,3,6-Galp residues than that (17.5%) in the newly solubilized CWP3. The results implied that relatively low proportions of the terminal arabinose residues were found in the newly solubilized CWP3 due to deficiency of 1,3,6-Galp residues. This confirmed the presence of single arabinofuranosyl residues as side chains of arabinogalactan in CWP3 of Japanese persimmon.

Methylation analysis of CWP3 (fresh) and the newly solubilized CWP3 (fraction 'a') revealed that $(1 \rightarrow 3,6)$-linked galactose was the main derivative in galactan side-chains. It was found that the depolymerized CWP3 (fraction 'b') contained markedly less $(1 \rightarrow 3,6)$-linked galactose than did any of the CWP3 (fresh) and 'a'

fractions. Deficiencies of $(1 \rightarrow 3)$- and $(1 \rightarrow 6)$-linked galactose residues were also found in depolymerized CWP3, indicating that the marked losses of $(1 \rightarrow 3,6)$-linked galactose residues from CWP3 occurred due to extensive degradation of main-chains of arabinogalactans. This implied losses of both types of sugars, arabinose (as terminal) and galactose from arabinogalactans in CWP3 of persimmon fruit during the sun-drying process.

Perhaps, in CWP3 of persimmon fruit, extensive degradation of galactans occurred due to β-galactosidase activity. β-galactosidase from different sources (Ali, Ng, Othman, Goh, & Lazan, 1998; Konno & Tsumuki, 1993) is able to degrade $(1 \rightarrow 3)/(1 \rightarrow 6)$-linked galactans. It is found that polyphenols inhibit β-galactosidase activity (Dick & Bearne, 1988). This activity can be enhanced in Japanese persimmon fruits during the sun-drying process due to insolubilization of tannins (polyphenols).

We previously reported (Asgar et al., 2003) that the total sugar contents of CWP3 (fresh) and the newly solubilized CWP3 (fraction 'a') were, respectively, 97.2% (22.1% of uronic acid and 75.1% of neutral sugar) and 97.6% (26.4% of uronic acid and 71.2% of neutral sugar). It was found that the sum of 1,5- and 1,3,5-linked arabinose residues (Table 1) was 23.3% and 43.4% of neutral sugar content, respectively, in CWP3 of fresh fruit and the newly solubilized CWP3 (or, 18.0% and 31.6% of total sugar content, respectively, in CWP3 of fresh fruit and the newly solubilized CWP3). In these fractions, the terminal arabinose residues originated from the branches of arabinogalactans and arabinans. In our previous paper (Asgar et al., 2003), it was shown that CWP3 (fresh) and the newly solubilized CWP3 had the same molecular weight distributions (105,000–329,000); depolymerization of CWP3 occurred during the sun-drying process; the molecular weight of a low-molecular-weight fraction of CWP3 (fraction 'c' from sun-dried persimmon) was less than 10,500; in fraction 'c', in addition to the presence of high proportions of uronic acid and rhamnose, intermediate proportions of arabinose (17.9% of total sugar content) and low proportions of galactose (8.0% of total sugar content) were found. Due to the presence of low proportions of galactose in fraction 'c', it is reasonable to assume that, in this fraction, arabinose residues originated predominantly from arabinans.

Extensive downshifts in molecular mass of CWP3 occurred during the sun-drying process and higher levels of arabinose were not found in the low molecular weight fraction ('c') than the sum of 1,5- and 1,3,5-linked arabinose residues in the high molecular weight fractions (CWP3 of fresh fruit and 'a'), indicating relatively high molecular weight of the arabinans present in CWP3 (fresh) and 'a' fractions compared to arabinans in fraction 'c'. Therefore, degradation of arabinans also occurred in CWP3 of persimmon fruit during the sun-drying process. Possibly, the activity of α-arabinosidase was increased in persimmon fruit during the sun-drying process due to insolubilization of tannins. α-arabinosidase is known (Rombouts et al., 1988) to split $(1 \rightarrow 3)$-, $(1 \rightarrow 2)$- and $(1 \rightarrow 5)$-α-L-arabinosyl linkages.

In our preceding paper (Asgar et al., 2003) it was shown that, during the sun-drying process, loss of arabinose occurred at a slower rate than galactose from CWP3; the sugar composition showed a relatively high arabinose/galactose ratio present in depolymerized CWP3 (fraction 'b') compared to that in either CWP3 (fresh) or the newly solubilized CWP3 (fraction 'a'). Methylation analysis gave similar results, as shown by the sugar composition. 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 papaya (Ali et al., 1998) and mango (Ali, Armugam, & Lazan, 1995).

During the sun-drying process, degradation of $(1 \rightarrow 3)/(1 \rightarrow 6)$-linked arabinogalactans and $(1 \rightarrow 5)$-linked arabinan side-chains occurred with downshifts in molecular mass of CWP3. A low-molecular-weight fraction of CWP3 (sun-dried persimmon) represented mainly the rhamnogalacturonan backbone of 'hairy' regions (Asgar et al., 2003). 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 arabinogalactans and arabinan side-chains.

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