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Cell-wall polysaccharides in the fruits of Japanese quince (*Chaenomeles japonica*): extraction and preliminary characterisation

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Abstract

The study of the cell-wall polysaccharides of the fruits of Japanese quince (*Chaenomeles japonica*) was achieved in two stages: (i) preparation of alcohol insoluble solids (AIS) from the different tissue zones and entire fruits of two genotypes and (ii) treatment of the AIS with extractants. The AIS represented an average of 32 g/100 g entire dry fruit and 4 g/100 g entire fresh fruit. A sequential extraction scheme allowed the separation of the cell-wall material into its major components (cellulose, pectins and hemicelluloses). The AIS was composed of 30 g pectins/100 g AIS, 8 g hemicelluloses/100 g AIS and 60 g cellulosic residue/100 g AIS. 100 g entire dry fruit (800 g entire fresh fruit) contained 11 g pectins, 3 g hemicelluloses and 18 g cellulosic residue. Pectins were mostly located in the flesh of the fruit, they were more efficiently extracted with hot dilute acid than with other extraction media and they had a high degree of methylation (DM) and a low degree of acetylation (DAc). Sequential extraction allows a first fractionation of the pectins. Hot dilute acid extracted pectins substituted with long Ara side-chains, whereas cold dilute alkali extracted pectins with more numerous but shorter side-chains. No difference was pointed out neither in the quantity of polysaccharides extracted from the two genotypes studied nor in the composition of these constitutive polysaccharides.

Keywords: *Chaenomeles*; Japanese quince; Alcohol insoluble solids; Cell-wall polysaccharides; Dietary fibres; Pectins; Extraction

1. Introduction

Japanese quince (*Chaenomeles japonica*) is one of the first shrub to bloom in spring and may continue to flower for a long time. Thus it is mostly grown as an ornamental plant. However, its fruit is known for its juice, aroma, fibres and pectins. The distinctive aroma (Lesinska, Przybylski, & Eskin, 1988) and the acid juice are the primary products after processing the fruits. The comparatively large amount of cell-wall polysaccharides in the fruits (Golubev, Kolechik, & Rigavs, 1990; Thomas, Crépeau, Rumpunen, & Thibault 2000) makes this crop also a potential source of dietary fibres and pectins. Juice and aroma processing residues may thus be valorised.

Our previous study on Japanese quince and flowering quince (*Chaenomeles speciosa*) (Thomas et al., 2000) showed that the fibre content of the fruits varied from 28 to 38 g fibres/100 g dry matter, depending on the genotype. Three homogeneous groups were distinguished: one with a low content of fibre, one with a medium content of fibre and one with a high content of fibre, the latter group being constituted by a single genotype of flowering quince. This variation must be taken into account when selecting varieties for fruit production and manufacture of fibre-rich products and pectins. Therefore, it would be interesting to know that if this variation can be ascribed to a variation in quantity or composition of pectins, hemicelluloses or cellulose. Thus, two genotypes of Japanese quince were chosen for a detailed study of the cell-wall polysaccharides. One was chosen in the group with a low content of fibres (RG822) and the second had a rather high content of fibres (NV9392). In this paper, cell-wall components have been carefully extracted from the entire fruits and different tissue zones and their chemical composition was determined.

2. Materials and methods

2.1. Plant material

The fruits were sampled from non-replicated genotypes (seedlings) in the collection kept at Balsgård-Department of Horticultural Plant Breeding, Swedish University of

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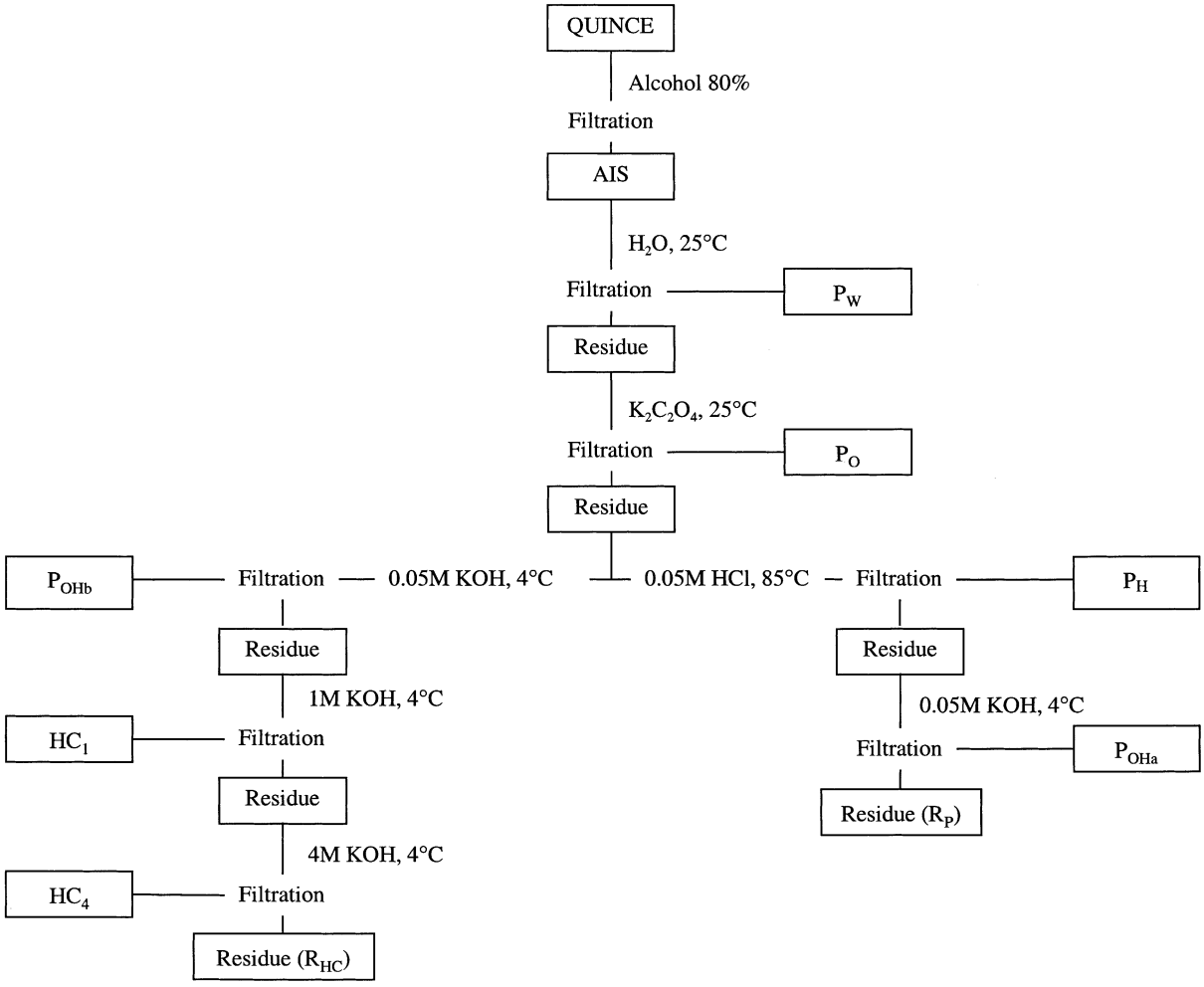

Fig. 1. Scheme for extraction of pectins and hemicelluloses fractions from Japanese quince cell-wall material.

Agricultural Sciences, Kristianstad, Sweden. The collection was gathered from partly domesticated populations in commercial orchards or from botanical gardens. All fruits were picked at the same developmental stage, when the seeds in the fruits had turned brown, indicating fruit maturity. After picking, seeds were removed and the fruits were sliced and freeze-dried.

The different tissues zones (skin, carpels, flesh) of freeze-dried genotypes NV9392 and RG822 were manually separated and weighed before further treatment.

2.2. Preparation of alcohol insoluble solids

Freeze-dried fruit materials (20, 7 and 1 g of flesh, carpels, skin, respectively, and 10 g of entire fruit) were cut into small pieces (~5 mm diameter) and homogenised in 800, 200, 100 and 400 ml boiling ethanol, respectively (final concentration of ethanol: 80%) in order to inactivate possible endogenous enzymes and remove alcohol-soluble solids. After boiling for 20 min, the residue was filtered through a G4 sintered glass (average pore diameter: 5–15 μm) and washed with 70% ethanol until a sugar-free extract was obtained (no sugar could be detected in the filtrate using a colorimetric reaction with phenol–sulfuric acid). The residue was washed successively with ethanol (96%, 3 times) and acetone (3 times), then air-dried overnight at 40 °C, vacuum-dried 12 h at 40 °C and weighed.

2.3. Sequential extraction

2.3.1. Pectins

Sequential extraction of pectins from alcohol insoluble solids (AIS) (Fig. 1) was based on the method described by Bertin, Rouau, and Thibault (1988). The extraction volume was adjusted to 60 ml/g of AIS and kept constant along the whole extraction sequence. Each extraction step was repeated 3 times. AIS was first treated with water at 25 °C for 30 min (pH was adjusted to 4.5 with 0.1 mol l^{-1} KOH). The slurry was filtered through a G4 sintered glass. Filtrates from the three consecutive extractions were pooled; if necessary, the pH was re-adjusted to 4.5 with 0.1 mol l^{-1} KOH or HCl and a filtration was performed through a 3 μm millipore membrane. The extract was concentrated, dialysed against deionised water at 4 °C

(until the conductivity of dialysate was less than 3 μS) and freeze-dried. The extract was named 'water-soluble pectins': P_W. The residue of P_W was then treated 3 times with 1% potassium oxalate (adjusted to pH 4.5 with 1 mol l^{-1} HCl) at 25 °C for 30 min. The slurry was filtered through a G4 sintered glass. Filtrates from the three consecutive extractions were pooled, treated as described earlier and named 'oxalate-soluble pectins': P_O. The residue of P_O was further treated with hot dilute hydrochloric acid (0.05 mol l^{-1}, 85 °C) for 30 min. After each extraction and prior to filtration, the pH of the slurry (≈ 1.3) was adjusted to 4.5 with 1 mol l^{-1} KOH. The slurry was filtered through a G4 sintered glass. Filtrates from the three consecutive extractions were pooled, treated as above and named 'dilute-acid-soluble pectins': P_H. The residue of P_H was then treated with cold dilute alkali (0.05 mol l^{-1}, 4 °C) for 30 min. The slurry was filtered through a G4 sintered glass. Filtrates from the three consecutive extractions were pooled, treated as earlier and named 'dilute-alkali-soluble pectins': P_{OHa}. The final residue (R_P) was washed with 50% ethanol until the conductivity of the filtrate was less than 10 μS. It was then dried by solvent exchange, stored one night at 40 °C and 12 h at 40 °C under vacuum and weighed.

2.3.2. Hemicelluloses

The extraction method was based on the method described by Selvendran and O'Neill (1987) and the extraction scheme can be seen in Fig. 1. The bulk of pectins was extracted as previously described but omitting the acid extraction; P_W, P_O and dilute-alkali-soluble pectins without a previous acidic extraction step (named P_{OHb}) were obtained. Solutions of increasing concentration of alkali (1 mol l^{-1} KOH and 4 mol l^{-1} KOH, both containing 20×10^{-3} mol l^{-1} $NaBH_4$) were then used to extract, under argon, the hemicelluloses named HC_1 and HC_4, respectively. The slurries were filtered through G4 sintered glasses. Filtrates from the three consecutive extractions were pooled; the pH was adjusted to 4.5 with 1 mol l^{-1} HCl and a filtration was performed through a 3 μm millipore membrane. The extracts were concentrated, dialysed against deionised water at 4 °C (until the conductivity of dialysate was less than 3 μS) and freeze-dried. The final residue (R_{HC}) was then washed with 50% ethanol until conductivity of the filtrate was lower than 10 μS. It was then dried by solvent exchange, stored one night at 40 °C and then one day at 40 °C under vacuum and weighed.

2.4. Chemical analysis

All values are on a dry weight basis.

2.4.1. Moisture

The moisture of the sugar-containing samples (freeze-dried fruits and extracts) was determined as the weight loss after vacuum drying at 40 °C until constant weight was obtained. The moisture of the AIS and residues was calculated as the weight loss after drying at 120 °C for 3 h.

2.4.2. Neutral sugar

All samples were hydrolysed in 1 mol l^{-1} H_2SO_4 (3 h, 100 °C) for measurement of individual neutral sugars (Englyst & Cummings, 1984), with an additional pre-treatment with 13 mol l^{-1} H_2SO_4 (1 h, 25 °C) for insoluble materials (AIS, R_P and R_{HC}) (Seaman, Moore, Mitchell, & Millet, 1954). Cellulosic glucose (Glc_C) was calculated as the difference between glucose amounts obtained with and without this pre-hydrolysis. The sugars were reduced to their corresponding alditols by adding 3 mol l^{-1} NH_3 containing $NaBH_4$ (10 mg). Reduction was performed 1 h at 40 °C. The excess of sodium borohydride was then destroyed by adding 2×0.05 ml glacial acetic acid. Acetylation was then performed with acetic anhydride (2 ml, 20 min at room temperature) in the presence of 1-methyl imidazole (0.2 ml) as a catalyst. Acetylation was stopped with 5 ml deionised water and the acetylated alditols were partitioned between dichloromethane (1.5 ml) and water. The aqueous phase was removed and two additional washings with 5 ml deionised water were performed. The samples were then analysed by GLC on an OV-225 (30 m × 0.32 mm) column at 200 °C, using hydrogen as carrier gas and a flame ionisation detector. Inositol was used as the internal standard.

2.4.3. Uronic acids

Insoluble samples were submitted to a 1 h prehydrolysis with 13 mol l^{-1} H_2SO_4 at 25 °C followed by a 3 h hydrolysis with 1 mol l^{-1} H_2SO_4 at 100 °C. Soluble samples (0.1 ml of 1 mg/ml pectin solutions) were treated with 0.05 mol l^{-1} NaOH (1.9 ml) for 30 min at room temperature and neutralised with 0.1 mol l^{-1} HCl (1 ml) before analysis. Uronic acids (UA) were determined by colorimetry (Blumenkrantz & Asboe-Hansen, 1973; Thibault, 1979). The difference in response of glucuronic acid (GlcA) and galacturonic acid (GalA) in presence and absence of tetraborate was used for their measurement (Renard, Crépeau, & Thibault, 1999). GlcA and GalA (Sigma–Aldrich, L'isle d'abeau, France) were used as standards.

2.4.4. Protein content

Nitrogen was determined by the semi-automatic Kjeldal method and protein content was estimated as N × 6.25.

2.4.5. Degrees of methylation and acetylation

The method used to measure methanol and acetic acid was described by Lévigne, Thomas, Ralet, Quemener, and Thibault (2001). Five milligram pectins were saponified during 2 h at room temperature in 1 ml of a 0.4 mol l^{-1} NaOH solution in 80% isopropanol. The supernatant obained by centrifugation at 7000*g* for 10 min was neutralised using a Maxi-Clean™ IC-H 0.5 ml device (Alltech) and analysed by HPLC equipped with a Merck–Superspher

Table 1
Distribution of weight and AIS in the different fruit tissue zones of two genotypes of Japanese quinces

Genotypes	RG822		NV9392	
	Weight[a]	AIS[a]	Weight[a]	AIS[a]
Entire fruit (determined)	100	29.2	100	35.0
Skin	7.9	45.7	9.2	48.0
Flesh	68.0	27.3	66.0	30.2
Carpels	24.1	51.4	24.8	61.6
Entire fruit (calculated)	100	34.6	100	39.6

[a] g/100 g dry fruit zone.

end-capped C18 cartridge (25×0.4 cm) column thermostated at 25 °C and equipped with a Merck C18 guard cartridge (0.4×0.4 cm). The eluant was 4×10^{-3} mol l^{-1} H_2SO_4 (0.7 ml min^{-1}). Maleic acid was used as an internal standard. The degrees of methylation (DM) and acetylation (DAc) were calculated as molar ratios of methanol and acetic acid, respectively, to GalA.

3. Results

3.1. Cell-wall material in the fruits

AIS content of the entire fruits and the different tissue zones can be seen in Table 1. The flesh was the major tissue, representing 68 and 66 g/100 g dry fruit of genotypes RG822 and NV9392, respectively. The skin corresponded to 8 and 9 g/100 g dry fruit and the carpels represented 24 and 25 g/100 g dry fruit of genotypes RG822 and NV9392, respectively. These results are similar to those described in our previous work (Thomas et al., 2000).

The tissues differed in their proportion of AIS (Table 1). The flesh contained less cell-wall material (27 and 30 g/100 g dry flesh of genotypes RG822 and NV9392, respectively) than the skin (46 and 48 g/100 g dry skin) or the carpels (51 and 62 g/100 g dry carpels). However, when extracted from entire fruit, most of the dietary fibres came from the flesh (54 and 50 g/100 g extracted fibres of genotypes RG822 and NV9392, respectively). The AIS content of the entire fruit was also experimentally determined and slightly higher values were obtained for the calculated than for the experimentally determined cell-wall material content of the entire fruit. This difference was probably due to multiplication of the experimental errors.

In the whole fruit, the AIS represented 29 g/100 g dry fruit and 35 g/100 g dry fruit for the genotypes RG822 and NV9392, respectively, in agreement with our previous work (Thomas et al., 2000). These results are much higher than those obtained for apple by Massiot, Baron, and Drilleau (1994) and Renard and Thibault (1991) where the AIS represented only 12.6 g/100 g dry matter of the entire fruit.

3.1.1. Neutral and acidic sugars in the cell-wall material

The composition of the AIS can be seen in Table 2. Total (acidic and neutral) sugars contents of the AIS determined from the entire fruits were 81 g total sugars/100 g AIS of RG822 and 75 g total sugars/100 g AIS of NV9392 (Table 2). For both genotypes, an average of 25 g total sugars/100 g dry matter in the whole dry fruit was obtained. Other components were the substituents of the pectins (acetic acid, methanol), proteins (8 and 5 g/100 g AIS of the entire fruits of genotypes RG822 and NV9392, respectively) and probably non-sugar cell-walls constituents such as polyphenols, lignin…. Protein content of the entire fruits were similar to those obtained for apple by Claye, Idouraine, and Weber (1996); Renard and Thibault (1991); Voragen, Timmers, Linssen, Schols, and Pilnik (1983).

In the entire fruits of genotypes RG822 and NV9392, respectively, acidic sugars represented 21 and 19 g/100 g AIS, and neutral sugars 60 and 56 g/100 g AIS. The carbohydrate polymers of the AIS were mostly pectic polysaccharides and glucans; in the AIS of entire fruits of genotypes RG822 and NV9392, respectively, UA represented 21 and 19 g/100 g AIS, Ara 10 and 9 g/100 g AIS, Gal 6 and 7 g/100 g AIS and Glc 32 and 29 g/100 g AIS. GlcA represented

Table 2
Composition of sugars and proteins in AIS (g/100 g AIS) of two genotypes of Japanese quince (Results were obtained from duplicates, std error <5%, Glc_C: cellulosic glucose, Glc_{HC}: non cellulosic glucose, UA: uronic acid, GlcA < 5%)

	Zone	Sugar composition									
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc_C	Glc_{HC}	UA	Prot
RG822	Entire (experimental)	1.2	0.7	10.2	8.0	1.8	6.1	30.0	2.0	21.0	8.0
	Skin	1.1	0.5	7.6	2.9	1.0	3.9	11.7	2.4	17.2	12.4
	Flesh	1.4	0.8	7.2	5.1	1.9	7.2	25.5	4.1	25.8	9.6
	Carpels	0.7	0.3	3.7	17.2	1.1	2.7	25.2	2.0	12.4	5.1
	Entire (calculated)	1.1	0.6	6.0	9.2	1.6	5.2	24.0	3.2	20.1	8.3
NV9392	Entire (experimental)	0.8	0.5	8.7	8.3	2.0	7.4	26.7	1.8	18.5	4.7
	Skin	0.8	0.4	6.8	3.1	1.2	3.9	11.3	2.2	16.7	9.6
	Flesh	1.1	0.8	7.6	5.4	2.9	9.6	25.1	4.2	23.6	7.6
	Carpels	0.7	0.4	5.2	14.9	1.5	4.3	22.6	3.0	13.9	3.5
	Entire (calculated)	0.9	0.6	6.6	8.8	2.2	6.9	22.6	3.5	19.1	6.2

Table 3
Distribution of cell-wall polysaccharides extracted from different fruit tissue zones of two genotypes of Japanese quince (g/100 g AIS)

	Zone	Pectin extraction					Hemicelluloses extraction			
		P_W	P_O	P_H	P_{OHa}	R_P	P_{OHb}	HC_1	HC_4	R_{HC}
RG822	Skin	2.5	1.5	14.6	6.3	71.0	7.5	3.8	3.6	58.0
	Flesh	7.7	1.9	19.9	8.2	42.0	21.7	5.3	4.1	54.0
	Carpels	2.6	1.9	11.9	4.5	73.0	10.8	4.6	2.8	76.0
	Entire fruit (calculated)	5.7	1.8	16.5	6.7	56.1	16.3	4.9	3.6	62.3
NV9392	Skin	3.0	2.2	18.3	3.6	69.0	10.2	3.8	3.1	43.0
	Flesh	7.4	1.9	20.1	8.1	51.0	14.8	5.8	4.7	62.0
	Carpels	3.0	1.5	10.6	2.8	77.0	6.8	3.8	1.4	81.0
	Entire fruit (calculated)	5.2	1.8	16.2	5.6	63.1	11.2	4.8	3.3	67.2

less than 5% of total acidic sugars. About 85% of the Glc was from cellulosic origin. These results were similar to those obtained for apple by Bittner, Burritt, Moser, and Street (1982) (acidic sugars: 18.7 g/100 g AIS, neutral sugars: 58.9 g/100 g the AIS).

The composition of the AIS was determined for each tissue-zone of the two genotypes. For the genotype RG822, the carbohydrate contents in the different zones represented from 48 to 79 g/100 g AIS, the skin being the less rich in carbohydrates and the flesh, the richest zone. Similar values were obtained for the genotype NV9392. These results were very close to those obtained previously (Thomas et al., 2000) and by Massiot and Renard (1997) for apple. The low level of carbohydrates in the epidermis zone may be attributed to the presence of cutin-like substances (Massiot et al., 1994) and proteins. Proteins represented 12 and 9 g/100 g AIS of the skin (for genotypes RG822 and NV9392, respectively), 10 and 8 g/100 g AIS of the flesh (for the same genotypes) and 5 and 4 g/100 g AIS of the carpels. Protein values were in the same range as those previously reported for apple by Massiot et al. (1994).

Most of the UA were located in the flesh of the fruit: per 100 g UA extracted from the entire fruit, 69 g were located in the flesh, 22 g in the carpels and 9 g in the skin of the genotype RG822. Results were similar for the genotype NV9392 (flesh: 62 g, carpels: 28 g, skin: 10 g). Moreover, Ara and Gal, the main non-cellulosic sugars were also mainly located in the flesh: per 100 g Ara or Gal extracted from the entire fruit, 65 and 74 g were located in the flesh (results were similar for the genotype NV9392: 58 and 70 g per 100 g Ara and Gal, respectively, were located in the flesh). These results indicated that pectins were probably mostly present in the flesh. The carpels were rich in hemicellulosic sugars; in the carpels of genotype RG822, Xyl and Glc represented, respectively, 17 and 25 g/100 g AIS, which was more than half of the total sugars (65 g sugars/100 g AIS). Similar results were obtained for genotype NV9392: Xyl and Glc represented 15 and 23 g/100 g AIS of the carpels, respectively, which was also more than half of the total sugars (67 g sugars/100 g AIS). Moreover most of the Xyl was present in the carpels: per 100 g Xyl extracted from the entire fruit, 67 and 65 g came from the carpels of genotypes RG822 and NV9392, respectively. Similar results were obtained for apple by Bittner et al. (1982) and for *Chaenomeles* species in our previous work (Thomas et al., 2000). The difference between the two genotypes only consisted in a higher quantity of cell-wall material in the genotype NV9392. Table 2 showed that calculated and experimental results were in rather good agreement.

3.2. Cell-wall polysaccharide content

The terms pectins, hemicelluloses and cellulosic residues are used in the text, even if the extracted polysaccharides were—at that stage of the study—not exactly identified. The extracts contents of the entire fruits and different tissue zones can be seen in Table 3.

A sequential procedure was used to extract pectic polysaccharides from the cell-walls. Hemicelluloses were extracted using another sequential method, which avoided the acidic extraction step in order to minimise degradation of the hemicelluloses. Alkaline degradation (peeling) of hemicelluloses was also minimised by using sodium borohydride (Selvendran & O'Neil, 1987). At each extraction step, the insoluble residues were not dried to avoid further irreversible collapse of cell-walls, which can hinder the following extractions. Therefore no intermediate yields could be calculated. The sum of the weight of the extracts and the final residues was $>80\%$, indicating that only few material was lost during extractions, except perhaps for the flesh and the skin for which the recoveries were lower, suggesting that some oligosaccharides or some non carbohydrate material may have been lost during dialysis of the extracts or during the successive extractions.

Yields of the different extracts can be seen in Table 3. The pectins ($P_W + P_O + P_H + P_{OHa}$) represented 31 and 29 g/100 g AIS for genotypes RG822 and NV9392, respectively. The hemicelluloses represented a very small proportion of total extracted polysaccharides: $HC_1 + HC_4 = 8.5$ and 8.1 g/100 g AIS of the entire fruits of the genotypes RG822 and NV9392, respectively. In our case, no precipitation of the hemicelluloses occurred during neutralisation of HC_1 and HC_4 with HCl. The cellulosic residues represented

Table 4
Composition of pectins extracted from different fruit tissue zones of two genotypes of Japanese quince

	Zone	Extract	Yield[a]	Sugar composition[b,c]							
				Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA[d]
RG822	Skin	P_W	2.5	0.7	0.3	9.2	1.5	0.7	5.0	3.5	54.1
		P_O	1.5	0.6	0.0	5.7	0.5	0.0	2.0	0.0	45.0
		P_H	14.6	1.1	0.2	19.4	0.7	0.1	5.6	1.7	38.3
		P_{OHa}	6.3	1.2	0.2	3.4	1.0	0.0	4.3	1.2	46.7
		P_{OHb}	7.5	0.8	0.0	5.9	0.5	0.0	2.4	0.4	54.6
	Flesh	P_W	7.7	0.9	0.2	7.6	0.9	1.4	4.7	3.0	49.0
		P_O	1.9	1.0	0.1	6.7	0.8	0.2	3.7	2.3	43.2
		P_H	19.9	1.2	0.2	12.6	1.0	0.3	6.3	1.3	54.8
		P_{OHa}	8.2	2.1	0.3	3.9	1.9	0.0	8.1	1.2	48.2
		P_{OHb}	21.7	1.3	0.2	7.7	0.6	0.0	5.5	0.4	52.2
	Carpels	P_W	2.6	0.8	0.2	8.4	1.1	1.6	4.2	2.8	61.5
		P_O	1.9	0.7	0.0	4.9	0.5	0.5	2.2	1.1	58.0
		P_H	11.9	1.4	0.2	15.9	0.7	0.0	5.4	1.1	48.4
		P_{OHa}	4.5	1.8	0.2	3.1	1.6	0.0	6.5	0.8	56.3
		P_{OHb}	10.8	1.2	0.1	6.9	0.3	0.0	3.0	0.1	60.7
NV9392	Skin	P_W	3.0	0.5	0.6	7.8	3.9	0.8	4.6	8.6	43.6
		P_O	2.2	0.6	0.2	6.1	1.3	0.5	3.1	2.6	58.0
		P_H	18.3	1.3	0.2	14.8	0.7	0.0	6.0	2.2	42.0
		P_{OHa}	3.6	1.6	0.2	3.5	1.4	0.0	6.2	1.7	40.1
		P_{OHb}	10.2	0.7	0.2	7.5	0.6	0.0	3.2	1.0	51.8
	Flesh	P_W	7.4	0.8	0.3	10.5	1.7	2.0	7.9	4.7	44.7
		P_O	1.9	0.7	0.2	5.0	0.8	0.8	4.6	2.1	54.2
		P_H	20.1	0.9	0.2	16.4	0.7	0.4	9.4	1.5	43.4
		P_{OHa}	8.1	1.6	0.3	5.5	0.8	0.0	13.6	0.7	55.4
		P_{OHb}	14.8	1.0	0.0	6.8	0.1	0.0	5.8	0.0	55.7
	Carpels	P_W	3.0	0.6	0.2	8.7	1.6	2.3	5.7	3.6	59.6
		P_O	1.5	0.5	0.0	3.5	0.4	0.2	2.2	0.9	66.0
		P_H	10.6	1.3	0.2	17.9	0.4	0.0	7.2	1.4	51.4
		P_{OHa}	2.8	2.0	0.2	2.7	1.1	0.0	8.2	0.8	50.7
		P_{OHb}	6.8	0.9	0.0	5.7	0.3	0.0	3.4	0.0	68.5

[a] g/100 g AIS.
[b] g/100 g extract.
[c] Results were obtained from duplicate, std error <5%.
[d] UA: uronic acids, GlcA < 5%.

more than 56 g/100 g AIS. No significant difference in pectin or hemicelluloses content was observed between the two genotypes, whereas, NV9392 had a higher cellulosic residue content than RG822 ($R_P = 56$ and 63 g/100 g AIS and $R_{HC} = 62$ and 67 g/100 g AIS for RG822 and NV9392, respectively).

On average, 66% of the pectins extracted came from the flesh, 10% from the skin and 24% from the carpels (Tables 1 and 3). Considering genotype RG822, $P_W + P_O + P_H + P_{OHa} = 37.7$, 20.9 and 24.9 g/100 g AIS in the flesh, carpels and skin, respectively. This result was in agreement with our previous work (Thomas et al., 2000). The cellulosic residue represented more than 70 g/100 g AIS in the skin and the carpels. It is likely that the amounts of pectins extracted from the skin, flesh or carpels differed slightly, depending on the extraction medium: on average 11, 7, 63 and 19% pectins were solubilised from the skin with water, oxalate, HCl and KOH, respectively; 20, 5, 53 and 22% pectins were solubilised from the flesh with water, oxalate, HCl and KOH, respectively, and 15, 9, 58 and 19% pectins were solubilised from the carpels with water, oxalate, HCl and KOH, respectively. Results were similar for genotype NV9392. Hot dilute acid extracted the highest quantity of pectins (an average of 58% of total extracted pectins were solubilised with the hot dilute acid), whereas oxalate solubilised the lowest quantities of pectins (an average of 7% of total extracted pectins were solubilised with oxalate). Water and cold dilute alkali solubilised 15 and 20% of total extracted pectins, respectively. Moreover, the total quantity of pectins extracted with water, oxalate, hot dilute acid and cold dilute alkali was higher than the total quantity of pectins extracted with water, oxalate and cold dilute alkali (when no acidic extraction was performed): $P_W + P_O + P_H + P_{OHa} = 38$ *g*/100 g AIS of the flesh for both RG822 and NV9392 and $P_W + P_O + P_{OHb} = 31$ and 24 g/100 g AIS of the flesh of RG822 and NV9392, respectively. These results indicated that pectins were more efficiently extracted when a step including hot dilute acid is used in the extraction scheme. This result was in agreement with studies on beets (Rombouts & Thibault, 1986) or grapes

Table 5
Degree of methylation and degree of acetylation of pectins extracted from different fruit tissue zones of two genotypes of Japanese quince

Zone	Degree of methylation[a]					Degree of acetylation[b]				
	P_W	P_O	P_H	P_{OHa}	P_{OHb}	P_W	P_O	P_H	P_{OHa}	P_{OHb}
RG822										
Skin	73	68	73	4	8	5	6	7	2	3
Flesh	71	64	61	7	7	3	3	3	1	1
Carpels	70	60	60	4	5	7	4	8	3	3
NV9392										
Skin	73	63	60	7	7	6	4	5	2	3
Flesh	80	65	59	4	<1	5	3	4	1	1
Carpels	74	62	57	4	5	9	5	8	3	3

[a] Std error $<5\%$.
[b] Std error $<10\%$.

(Saulnier & Thibault, 1987) but disagreed with the results obtained for carrots (Massiot, Rouau, & Thibault, 1988) or apple (Massiot et al., 1994).

3.3. Composition of the fractions

3.3.1. Pectins

Composition of the pectin extracts (P_W, P_O, P_H, P_{OHa} and P_{OHb}) can be seen in Table 4. Some GlcA was found to be present in some pectins (Renard et al., 1999). In our samples, less than 5 g GlcA/100 g UA was detected in the extracts.

In all the extracts from each tissue-zone of the two genotypes, UA, Rha, Ara and Gal were the main sugars detected, indicating that pectins were the main polysaccharides in the extracts. Neutral sugars represented from 7.7 to 29.6 g/100 g extract and acidic sugars from 38.3 to 68.5 g/100 g extract. Total neutral and acidic sugars in the pectins represented an average of 70 g/100 g extract. Other constituents may be substituents of the pectins (methanol, acetic acid) or proteins. Pectin extracts from the carpels seemed to contain less non-sugar components (74 g sugars/100 g extract, on average) than the flesh (70 g sugars/100 g extract, on average) or the skin (65 g sugars/100 g extract, on average). This result was in agreement with the high protein contents of the AIS of the skin (see Table 2).

Independently of the tissue-zone, the hot dilute acid solubilised material rich in Ara and Gal. Approximately 80 and 60% of the Ara and of the Gal, respectively, were solubilised at this step. This suggested that these pectins (P_H) have more or longer side-chains than the other (P_W, P_O, P_{OHa}), in agreement with the results described by Massiot et al. (1994). It has been claimed (Selvendran, Stevens, & O'Neill, 1985) that P_O may originate from the middle lamella, whereas P_H come from the primary cell-wall. In all the extracts from each tissue-zone of the two genotypes, the rhamnose to uronic acids ratios (Rha/UA) were not very high (0.02 on average), indicating pectins had a low proportion of regions carrying side-chains, even if the Rha content of the fraction may be underestimated (see Section 4). These ratios increased from oxalate- or water-soluble pectins to dilute-acid- or dilute-alkali-soluble pectins (P_{OHa}). This result was in agreement with those found for apple by Renard, Voragen, Thibault, and Pilnik (1990). It suggested that dilute-acid-soluble pectins were more branched than water- or oxalate-soluble pectins, but less branched than dilute alkali-soluble pectins (P_{OHa}). As alkali-soluble pectins contained lower amounts of neutral sugars than acid-soluble pectins, dilute alkali-soluble pectins may be substituted with many short neutral-sugar-side-chains, whereas acid-soluble pectins may have fewer but longer side-chains.

The rhamnose to UA ratio was the highest for P_{OHa}, whatever the tissue zone whereas it was intermediate for P_{OHb}. Ratios could be sorted as such: $P_{OHa} > P_H > P_{OHb} > P_W > P_O$, suggesting that pectins extracted with dilute alkali after an acidic treatment (POHa) were more branched than those extracted with dilute alkali alone (P_{OHb}). Dilute acid may have degraded side-chains, thus giving dilute alkali the possibility to liberate pectins with short side chains. Without an acidic treatment, only less branched pectins can be liberated. The yield of pectins extracted with dilute alkali alone (P_{OHb}) was approximatively one half of that obtained with hot dilute acid followed by a dilute alkali extraction ($P_H + P_{OHa}$). Moreover, alkali treatments, when achieved after an acidic extraction, yield rather low amount of polysaccharides (from 2.8 to 8.2 g/100 g AIS). These results indicated that acid-labile linkages seemed to play a major role for the extraction of pectins. More Ara was extracted by dilute alkali alone (P_{OHb}) than by dilute alkali following an acidic extraction (P_{OHa}). This was probably due to an acidic hydrolysis of arabinose moieties to small oligomers. On the contrary, Xyl and Gal were more efficiently extracted in P_{OHa} than in P_{OHb}.

Pectins from the flesh, carpels and skin seemed to have similar degrees of branching as suggested by the constancy of the rhamnose to UA ratios. Pectins from the carpels had a slightly higher proportion of GalA than those from the flesh or the skin. Massiot et al. (1994) found that pectins from the carpels of apple also had a higher GalA content than those from the flesh and that pectins from the carpels and those from the skin had a similar GalA content.

Tables 3 and 4 showed that the AIS of entire fruits of genotypes RG822 and NV9392, not only contained the same amount of pectins ($P_W + P_O + P_H + P_{OHa} =$ 31 and 29 g/100 g AIS, respectively), but also that the composition of these pectins was very similar (68 and 71 g sugars/100 g extract, respectively). Thus, the difference observed in dietary fibre contents of the two genotypes cannot be attributed to the pectins.

The DM and acetylation of pectins can be seen in Table 5. The degree of acetylation was calculated, assuming that only pectins were acetylated.

Pectins had a high DM (range: 59–80) and a low DAc (range: 0–9). As anticipated, pectins extracted with alkali had low DM (<8) and low DAc (<3). There was no

Table 6
Composition of hemicelluloses extracted from different fruit tissue zones of two genotypes of Japanese quince

	Zone	Extract	Yield[a]	Sugar composition[b,c]							
				Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA[d]
RG822	Skin	HC_1	3.8	0.7	1.6	6.1	12.4	3.2	7.1	14.9	3.2
		HC_4	3.6	0.6	0.9	4.4	4.7	4.5	5.3	9.3	2.7
	Flesh	HC_1	5.3	0.5	3.3	4.2	22.0	2.0	10.7	29.3	2.6
		HC_4	4.1	1.0	1.7	4.5	9.9	13.7	10.8	22.5	3.5
	Carpels	HC_1	4.6	0.6	1.7	3.2	36.0	2.9	6.8	16.2	5.4
		HC_4	2.8	0.6	1.7	2.7	16.8	9.9	8.4	20.1	2.5
NV9392	Skin	HC_1	3.8	0.5	1.9	4.2	16.3	4.5	8.5	20.6	2.3
		HC_4	3.1	0.6	0.6	4.8	6.9	7.0	6.9	13.2	3.2
	Flesh	HC_1	5.8	0.4	3.1	4.2	25.6	3.0	11.1	29.5	2.7
		HC_4	4.7	0.8	1.6	4.5	10.0	12.8	11.1	21.3	2.3
	Carpels	HC_1	3.8	0.5	1.7	2.8	32.0	3.5	6.6	17.1	4.8
		HC_4	1.4	0.6	1.8	2.8	24.0	6.1	7.5	17.8	3.7

[a] g/100 g AIS.
[b] g/100 g extract.
[c] Results were obtained from duplicate, std error <5%.
[d] UA: uronic acids, GlcA < 5%.

difference between the two genotypes. Some unknown peaks were present on the HPLC chromatograms while determining the DM and DAc (results not shown). They were eluted at the same retention times than quinic and malic acids, the main organic acids contained in the fruits of Japanese quince (Rumpunen, 1996).

3.3.2. Hemicelluloses

Compositions of the different extracts of hemicelluloses can be seen in Table 6. Some GlcA was found to be present in some hemicelluloses (Selvendran & O'Neil, 1987). In our samples, less than 5 g GlcA/100 g UA was detected in the hemicelluloses. The main constitutive sugars of the extracts were Xyl, Man, Gal and Glc. These sugars are representative of hemicellulosic fractions. Total acidic sugars represented less than 5 g/100 g extract and GlcA amount was less than 5% of total UA. Total neutral sugars represented more than 50 g/100 g extracts, except in the skin where they represented only 46 g/100 g extract, on average. Some non-carbohydrate constituents, such as proteins or polyphenols, may also be present in the extracts from the skin.

Hemicelluloses extracted with 1 mol l^{-1} KOH and those extracted with 4 mol l^{-1} KOH differed by their monosaccharides constituents. HC_1 contained high proportions of Xyl, probably coming from xylans, whereas HC_4 contained high proportions of Man, probably coming from mannans. These results were similar to those obtained for the cauliflower by Femenia, Waldron, Robertson, and Selvendran (1999).

Table 7
Composition of cellulosic residues extracted from different fruit tissue zones of two genotypes of Japanese quince

	Zone	Extract	Sugar composition[a,b]							
			Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA[c]
RG822	Skin	R_P	0.5	0.3	1.8	4.2	0.9	2.3	17.6	5.9
		R_{HC}	0.9	0.5	8.1	4.6	0.6	3.6	20.8	15.5
	Flesh	R_P	0.8	0.9	1.6	7.8	2.8	5.9	49.6	8.6
		R_{HC}	1.0	0.8	4.1	5.2	1.3	5.7	44.7	11.3
	Carpels	R_P	0.5	0.3	1.0	16.3	1.8	2.3	36.6	5.9
		R_{HC}	0.8	0.3	5.1	13.7	1.1	3.3	37.6	9.1
NV9392	Skin	R_P	0.3	0.2	1.1	3.1	1.1	1.4	14.8	4.3
		R_{HC}	0.8	0.4	7.8	4.2	0.9	3.9	20.6	14.7
	Flesh	R_P	0.7	0.9	1.9	8.3	3.5	7.7	39.6	5.4
		R_{HC}	1.0	0.6	6.0	4.4	2.3	9.1	40.0	15.4
	Carpels	R_P	0.4	0.2	0.9	19.9	1.3	2.2	30.0	5.7
		R_{HC}	0.5	0.1	3.2	20.3	0.7	2.3	28.8	7.4

[a] g/100 g extract.
[b] Results were obtained from duplicated, std error <5%.
[c] UA: Uronic Acids, GlcA < 5%.

Hemicelluloses still contained some GalA (from 2.3 to 5 g/100 g extract) and Rha (from 0.4 to 1 g/100 g extract), indicating that some pectic material remained associated with the hemicelluloses.

The flesh contained the highest quantity of hemicelluloses. There was no major difference between the hemicelluloses from the different tissue zones of the fruit; however, the hemicelluloses from the carpels contained more Xyl than those from the flesh. Those from the flesh contained more Glc than those from the carpels and from the skin.

Again, no difference in the composition nor quantity of the hemicelluloses was observed between the two genotypes.

3.3.3. Cellulosic residues

Monosaccharides composition of the cellulosic residues can be seen in Table 7.

The residue was mostly constituted of neutral sugars and particularly Glc. Total neutral and acidic sugars represented only 34 and 55 g/100 g R_P and R_{HC}, respectively, for the skin of RG822 and 26 and 53 g/100 g R_P and R_{HC}, respectively, for the skin of NV9392. The residue of the skin also contained many non-carbohydrate constituents.

Some GalA (from 4.3 to 15.5 g/100 g extract) were still remaining in the residues showing that pectins are still present in the final residues. The composition of R_P was very close to that of R_{HC}. However, R_{HC} contained more uronic acids (12 g UA/100 g extract, on average) than R_P (6 g UA/100 g extract, on average) underlining the fact that the acidic extraction step was the most efficient to extract the pectins.

There was no major difference between the cellulosic residues from the different tissue zones of the fruit; however, the same differences noticed for the hemicelluloses can also be shown for the cellulosic residues (cellulosic residues from the carpels contained more Xyl than those from the flesh and those from the skin. Cellulosic residues from the flesh contained more Glc than those from the carpels and those from the skin), indicating that some hemicelluloses may be linked to the cellulose.

The different tissue-zones of genotype NV9392 contained higher amounts of cellulosic residue than the different tissue-zones of genotype RG822. However, residues from the different tissue-zones of genotype NV9392 had lower contents of polysaccharidic material than residues from the different tissue-zones of genotype RG822. Once again, the difference in dietary fibre contents of the two genotypes is not due to their constitutive polysaccharides.

4. Discussion and conclusions

The study of the cell-wall polysaccharides of japanese quince was achieved in two stages: (i) preparation of AIS and (ii) treatment of the AIS with sequential extraction media. Preparation of AIS is commonly used to isolate cell-wall materials (Selvendran et al., 1985). Although this method has some drawbacks, it is considered suitable for fruits and vegetables that contain low amounts of starch, intra-cellular proteins and polyphenols (Selvendran & O'Neil, 1987; Selvendran et al., 1985). The proteins in the AIS (4–12%) could be ascribed to cell-wall proteins or co-precipitated intracellular proteins. The AIS represented an average of 32 g/100 g entire dry fruit and 4 g/100 g entire fresh fruit.

The present extraction scheme (Fig. 1) allowed the separation of the cell-wall material into its major components (mainly cellulose, pectins and hemicelluloses). The AIS of entire fruits was composed of 30 g pectins/100 g AIS, 8 g hemicelluloses/100 g AIS and 60 g cellulosic residue/100 g AIS. 100 g entire dry fruit (800 g entire fresh fruit) contained 11 g pectins, 3 g hemicelluloses and 18 g cellulosic residue. It was also important to note that pectins were mostly located in the flesh of the fruits.

Sequential extraction is generally used for the fractionation of the pectins. Following the water extraction, Ca^{++} chelators ethylenediamine tetraacetate (EDTA) or cyclohexane-*trans*-1,2 diamine tetraacetate (CDTA) are often used to extract pectins. However, it was shown (Mort, Moerschbacher, Pierce, & Maness, 1991) that CDTA or EDTA may remain associated with the pectins, even after extensive dialysis against distilled water. Thus, oxalate was used to overcome this problem. Water and oxalate (pH 4.5, at 25 °C) solubilised a low amount of pectins (6 and 2 g/100 g AIS, respectively). These extraction conditions were not degradative and pectin structure must have been preserved from any degradation, and particularly from β-eliminations. The following extractions (hot dilute acid and cold dilute alkali) are degradative conditions and pectin structure may have been modified. The highest quantity of pectins (17 g/100 g AIS) was extracted with 0.05 mol l^{-1} HCl, 85 °C. These conditions are known to break some covalent linkages, especially those involving Ara and Rha residues. Subsequent treatment with alkali liberated some additional pectins (6 g/100 g AIS), probably through limited β-elimination reactions of methyl galacturonate residues. Simultaneously, most of the methyl ester and acetyl groups were removed. At this stage, not all the pectins are removed from the AIS, its content of UA being still 5 and 7 g/100 g AIS of genotypes NV9392 and RG822, respectively. These results are similar to those obtained for sugar beet pulp (Bertin et al., 1988; Rombouts & Thibault, 1986).

Pectins extracted under non alkaline conditions (P_W, P_O and P_H) had a high DM and a low degree of acetylation. Pectins extracted by a chelating agent are believed to be complexed mainly with Ca^{++} (Selvendran et al., 1985). However, oxalate-soluble pectins often have a high content of methyl ester groups (Renard et al., 1990; Ros, Schols, & Voragen, 1998) and Table 5 shows that the DM of P_O was high (>60%). A complexation through cation ions would only be possible if the methoxyl groups were block-esterified, which would have to be confirmed (Ralet, Dronnet, Buchholt, & Thibault, 2001).

Recovery of each sugar during the extractions was close to 100%. However, Ara and Rha had a substantially lower yield (respectively 74 and 73%, on average). Determination of the sugar composition of polysaccharides involved an acidic hydrolysis. The various stabilities of the monosaccharides liberated, as well as those of the different linkages involved would have required different optimum conditions of hydrolysis: a long and harsh hydrolysis step would destroy some of the Ara whereas a short and mild hydrolysis would underestimate the Rha. Thus, the conditions should ideally be determined and optimised for each material. Our conditions were optimised for the AIS (Thomas et al., 2000) and applied, as a compromise, to all samples. Therefore, Rha and Glc may be underestimated in all the extracts. Ara was also underestimated due to the extraction method that includes an acidic step. Indeed, below pH 3, a hydrolysis occurred of the labile arabinofuranoside linkages by which many of the neutral polysaccharides are joined to the rhamnogalacturonan backbone (Selvendran et al., 1985). Some Ara may thus be eliminated during dialysis of the extracts.

Genotype NV9392 had a higher AIS content than genotype RG822. However, no difference was pointed out neither in the quantity of polysaccharides extracted from the AIS of the two genotypes nor in the composition of these constitutive polysaccharides. The difference in AIS content may thus be attributed to non polysaccharidic material such as polyphenols or organic acids that may be 'trapped' in the cell-walls. However this study involved only two genotypes. More genotypes would have to be analysed to confirm this result. The interest of these first results on cell-wall polysaccharides of Japanese quince justify that further studies should be devoted to purify its cell-wall polysaccharides, especially pectins and determine their fine structure.

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