

# Dietary Fibre and Cell-wall Polysaccharides in Chaenomeles Fruits

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## SUMMARY

In this paper, research on dietary fibre and cell-wall polysaccharides in chaenomeles fruits is reported and summarised.

The dietary fibre in fruits of 12 genotypes of Japanese quince (*Chaenomeles japonica*) and 1 genotype of flowering quince (*C. speciosa*) was prepared using two different methods: the Alcohol Insoluble Solid (AIS) method; and the AOAC method for total as well as for soluble and insoluble fibre. The two methods resulted in significantly different estimates, however, no interaction was found between the methods and the genotypes studied. For content of total dietary fibre, three main groups were distinguished, one containing a low amount of fibre (3 genotypes, 28–30 g/100 g dry matter); one containing a moderate amount of fibre (9 genotypes, 30–36 g/100 g dry matter) and an isolated genotype (*C. speciosa*) that contained a high amount of fibre (38 g/100 g dry matter). The amount and the nature of monomeric sugars in the constituent polysaccharides of the fibre were determined after total hydrolysis of the AIS and the TDF (Total Dietary Fibre). The fibre contained mostly pectic and cellulosic polysaccharides.

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, 8 g hemicelluloses and 60 g cellulosic residue/100 g AIS. In 100 g entire dry fruit (800 g entire fresh fruit) there were 11 g pectins, 3 g hemicelluloses and 18 g cellulosic residue. Pectins were mostly located in the flesh of the fruit. Pectins were more efficiently extracted with hot dilute acid than with other extraction media. Pectins had a high degree of methylation (DM) and a low degree of acetylation (DAc). No difference was found in the quantity of polysaccharides extracted from two Japanese quince genotypes, or in the composition of these constituent polysaccharides.

The physico-chemical properties of pectins extracted from two genotypes of Japanese quince were studied. On average, the fruits contained 11 g pectins/100 g dry fruit corresponding to 1.4 g pectins/100 g fresh fruit. Pectins were sequentially extracted, and the cells from the flesh of the fruits were observed with a confocal laser scan microscope. Although the dilute acid conditions were the most efficient for extraction of pectins, pectins extracted by water or potassium oxalate had higher (> 600 ml/g) intrinsic viscosities than pectins extracted by dilute acid (< 400 ml/g). Anionic exchange chromatography was performed on the acid-extracted pectins. The pectins were composed of four populations, the first being mainly composed of arabinans, the second of homogalacturonans and the third of rhamnogalacturonans. The composition of the fourth population differed depending on the genotype studied.

## INTRODUCTION

In this paper, recently published research (Thomas *et al.* 2000, Thomas 2001, Thomas & Thibault 2002) on dietary fibre and cell-wall polysaccharides in chaenomeles fruits is summarised and discussed.

Because of the potential health benefits of foods rich in dietary fibre (*e.g.* protection against cardiovascular diseases, diabetes, obesity, colon cancer) many Western countries have recommended an increased consumption of such food as part of their dietary guidelines. This knowledge has also resulted in efforts to find new sources of dietary fibre and more research has been devoted to investigations aimed at revealing fibre composition.

A potential new fruit crop presently being domesticated is Japanese quince (*Chaenomeles japonica*) (Rumpunen 2002). The distinctive aroma (Lesinska *et al.* 1988) and the acid juice are the primary products after processing of the fruits. However, the comparatively large amount of dietary fibre and soluble polysaccharides in the fruits (Golubev *et al.* 1990) also makes this crop a promising candidate for the manufacture of dietary fibre-containing food products and pectins. Juice and aroma processing residues thus need to be evaluated.

In the beginning of a breeding programme, it is important to obtain information on the variation in different quality characteristics of the fruits, which in the end will enable efficient selection of varieties. Neither the variation in content of dietary fibre between genotypes nor the composition and distribution of the constituent monomeric sugars of the fibre has previously been investigated for chaenomeles fruits. We studied the total dietary fibre content in 13 genotypes using two different methods: the Alcohol Insoluble Solid (AIS) method and the Association of Official Analytical Chemists (AOAC) method (Prosky *et al.* 1988, AOAC Methods 1990, 1992). The amount and the nature of monomeric sugars in the constituent polysaccharides were determined and the composition of the different parts of the fruit was analysed (Thomas *et al.* 2000).

Based on the fibre content of the fruits, homogeneous groups of genotypes were revealed. Such a variation could be taken into account when selecting varieties for fruit production and manufacture of fibre-rich products and pectins. Therefore, it was interesting to find out if this variation could be ascribed to a variation in quantity or composition of pectins, hemicelluloses or cellulose (Thomas & Thibault 2002). Furthermore, due to their gelling properties, pectins are often used as an additive in the food industry (E440). It was therefore of interest also to determine some of the chemical and physico-chemical properties of the pectins in Japanese quince.

In the genus *Chaenomeles*, the fruit develops from an inferior ovary, where the hypanthium is completely fused to the ovary. The fleshy part of the fruit corresponds to the hypanthium and the fruit is thus a “false fruit”. Using the terminology epicarp, mesocarp and endocarp could therefore be misleading. The common vocabulary: skin, flesh, and carpels, is therefore used in our research when referring to the different parts of the fruit.

## MATERIALS AND METHODS

### Plant material

The fruits were sampled from non-replicated genotypes (selected seedlings) in the collection kept at Balsgård–Department of Horticultural Plant Breeding, Swedish University of 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 from 12 genotypes of Japanese quince (NV1944, RG822, NV1410, NV1718, NV1597, D3122, NV1473, NV19108, NV152, D694, NV9392, NV1511), and one genotype of flowering quince (*C. speciosa*, RG840) were sliced and freeze-dried. For genotypes D694, NV1511, NV9392 and RG822, the different tissue zones (skin, carpels and flesh) were manually

separated from the freeze-dried fruits and weighed for a detailed study of the content and composition of polysaccharides in each tissue zone. Another batch of entire fruits of genotypes RG822 and NV9392 was frozen immediately after picking.

### Preparation of alcohol insoluble solids (AIS)

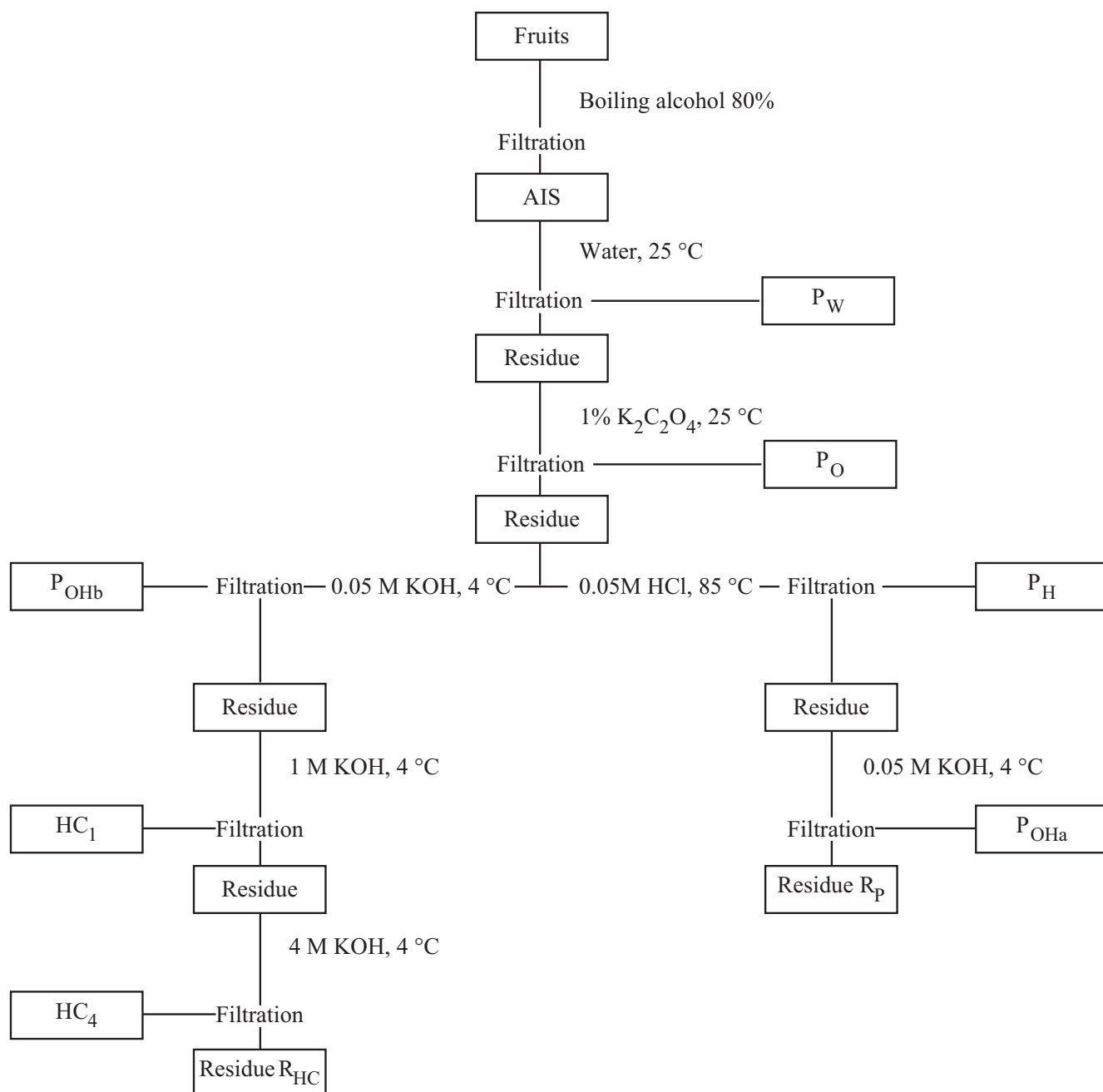
Freeze-dried fruit material (20 g flesh, 7 g carpels, 1 g skin and 10 g of entire fruit) was cut into small pieces (~5 mm diameter). It was then homogenized in 800, 200, 100 and 400 ml boiling ethanol, respectively (at an 80% final concentration of ethanol) in order to inactivate possible endogenous 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  $\mu\text{m}$ ) and washed with 70% ethanol until a sugar-free extract was obtained. The residue was washed successively with 96% ethanol (3 times) and acetone (3 times), then air-dried overnight at 40 °C, vacuum-dried for 12 h at 40 °C, and weighed (AIS).

### Preparation of dietary fibre

Freeze-dried fruit material was cut into small pieces (~0.5 mm diameter) in liquid nitrogen. The dietary fibre was measured gravimetrically after removing the non-fibre constituents by enzymatic degradation as described in the AOAC method. Duplicates (2 x 1 g) in a phosphate buffer (0.08 M, pH 6) were analysed for soluble (SDF) and insoluble (IDF) dietary fibre. Samples were treated with a thermostable  $\alpha$ -amylase (Termamyl 120L, Novo Nordisk A/S, Denmark) and then digested with a protease (P 5380, Sigma Aldrich, France) and an amyloglucosidase (E.C. 3213, Megazyme, Ireland) to remove protein and starch, respectively. SDF and IDF were separated by filtration (G4 funnel). The retentate (IDF) was first dried by solvent exchange, then under vacuum overnight at 40 °C. The filtrate containing SDF was recovered after alcoholic precipitation with 4 volumes of 96% ethanol, then dried by solvent exchange under vacuum overnight at 40 °C. A control was performed following the same procedure. An estimated content of Total Dietary Fibre (TDF) was then obtained as the combined values of SDF and IDF after corrections for proteins and the control had been made.

### Sequential extraction of pectins

Sequential extraction of pectins from AIS (Figure 1) was based on the method described by Bertin *et al.* (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 three times. AIS was first treated with water at 25 °C for 30 min (pH was adjusted to 4.5 with 0.1 M 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 M KOH or HCl, and a filtration was performed through a 3  $\mu\text{m}$  millipore membrane. The extract was concentrated, dialysed against deionised water at 4 °C (until the conductivity of dialysate was less than 3  $\mu\text{S}$ ) and freeze-dried. The extract was named 'water-soluble pectins':  $P_w$ . The residue of  $P_w$  was then treated three times with 1% potassium oxalate (adjusted to pH 4.5 with 1 M 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 above and named 'oxalate-soluble pectins':  $P_o$ . The residue of  $P_o$  was further treated with hot dilute hydrochloric acid (0.05 M, 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 M 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 M, 4 °C) for 30 min. The slurry was filtered through a G4 sintered glass. Filtrates from the three consecutive extractions were pooled, treated as above 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  $\mu\text{S}$ . It was then dried by solvent exchange, stored overnight at 40 °C, vacuum-dried for 12 h at 40 °C, and then weighed.



**Figure 1.** Scheme for extraction of pectins (P) and hemicelluloses (HC) fractions from Japanese quince cell-wall material.

### Sequential extraction of hemicelluloses

The extraction method was based on the method described by Selvendran & O'Neill (1987) and the extraction scheme is shown in Figure 1. The bulk of pectins was extracted as previously described but omitting the acid extraction, thus  $P_W$ ,  $P_O$ ,  $P_{OHb}$  ('dilute-alkali-soluble pectins without a previous acidic extraction step' were named  $P_{OHb}$ ) were obtained. Solutions of increasing concentration of alkali (1 M KOH and 4 M KOH, both containing 0.020 M  $NaBH_4$ ) were then used to extract under argon the hemicelluloses named  $HC_1$  and  $HC_4$ . 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 M HCl and a

filtration was performed through a 3  $\mu\text{m}$  millipore membrane. The extracts were concentrated, dialysed against deionised water at 4 °C (until the conductivity of dialysate was less than 3  $\mu\text{S}$ ) and freeze-dried. The final residue ( $R_{\text{HC}}$ ) was then washed with 50% ethanol until conductivity of the filtrate was lower than 10  $\mu\text{S}$ . It was then dried by solvent exchange, stored one night at 40 °C, one day at 40 °C under vacuum, and then weighed.

### Chemical analysis

All values are reported on a dry weight basis, and analyses were performed in duplicate.

### Moisture

The moisture of the freeze-dried fruits and of the extracts was determined as the weight loss after vacuum drying at 40 °C until a 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.

### Protein content

Nitrogen was determined by the semi-automatic Kjeldal method and protein content was estimated as  $\text{N} \times 6.25$ .

### Ash content

Ash was determined as the weight loss after incineration overnight at 550 °C followed by 1 h at 900 °C.

### Analysis of uronic acids and neutral sugar by colorimetry

Insoluble samples were submitted to a 1 h prehydrolysis with 13 M  $\text{H}_2\text{SO}_4$  at 25 °C (Seaman *et al.* 1954) followed by a 3 h hydrolysis with 1 M  $\text{H}_2\text{SO}_4$  at 100 °C. Soluble samples (0.1 ml of 1 mg/ml pectin solutions) were treated with 0.05 M NaOH (1.9 ml) for 30 min at room temperature and neutralised with 1 ml 0.1 M HCl before analysis. Uronic acids were determined as ‘anhydrogalacturonic’ acid by colorimetry (Blumenkrantz & Asboe-Hansen 1973, Thibault 1979). The difference in response of glucuronic acid (GlcA) and galacturonic acid (GalA) in the presence and absence of tetraborate was used for their measurement (Renard *et al.* 1999). GlcA and GalA (Sigma-Aldrich, L’isle d’abeau, France) were used as standards. Neutral sugars were determined by colorimetry with orcinol (3,5-dihydroxytoluene) after correction for the interference due to uronic acids (Tollier & Robin 1979). Ara was used as a standard.

### Analysis of neutral sugars by GLC

All samples were hydrolysed in 1 M  $\text{H}_2\text{SO}_4$  (3 h, 100 °C) for measurement of individual neutral sugars (Englyst & Cummings 1984), with an additional pre-treatment with 13 M  $\text{H}_2\text{SO}_4$  (1 h, 25 °C) for insoluble materials (AIS,  $R_p$  and  $R_{\text{HC}}$ ) (Seaman *et al.* 1954). The sugars were reduced to their corresponding alditols by adding 3 M  $\text{NH}_3$  containing  $\text{NaBH}_4$  (10 mg). Reduction was performed for 1 h at 40 °C. The excess of sodium borohydride was then destroyed by adding 2 x 0.05 ml glacial acetic acid. Acetylation was 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 deionized 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 deionized water were performed. The samples were then analysed by GLC on an OV-225 (30 m x 0.32 mm) column at 200 °C, using hydrogen as the carrier gas and a flame ionization detector. Inositol was used as the internal standard.

### Degrees of methylation and acetylation

The method used to measure methanol and acetic acid was described by Lévigne *et al.* (2002). 5 mg pectins were saponified for 2 h at room temperature in 1 ml of a 0.4 M NaOH solution in 80% isopropa-

nol. The supernatant obtained by centrifugation at 7000 g for 10 min was neutralized using a Maxi-Clean™ IC-H 0.5 ml device (Alltech) and analysed by HPLC equipped with a Merck-Superspher end-capped C18 cartridge (25 x 0.4 cm) column thermostated at 25 °C and equipped with a Merck C18 guard cartridge (0.4 x 0.4 cm). The eluent was 0.004 M H<sub>2</sub>SO<sub>4</sub> (0.7 ml/min). 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 galacturonic acid.

### Anion exchange chromatography

Solutions (50 ml) of pectins (2 mg/ml) were loaded on a column (37 x 2.6 cm) of DEAE Sepharose CL-6B (Amersham Pharmacia, Uppsala, Sweden) equilibrated and eluted at room temperature with 500 ml of a 0.05 M sodium succinate buffer (pH 4.5) at 1.27 ml/min. Pectic material bound to the gel was then eluted by increasing the ion strength of the buffer. A linear gradient (1500 ml) from 0 to 0.4 M NaCl in 0.05 M sodium succinate buffer was applied to the column. The column was then washed with 380 ml of 0.4 M NaCl in 0.05 M sodium succinate buffer. The fractions (12.7 ml) were assayed colorimetrically for galacturonic acid and neutral sugars. The purified pectin fractions were extensively dialysed against deionised water and freeze-dried.

### High-performance size-exclusion chromatography (HPSEC) and viscosity measurements

Pectins (5 mg/ml) were solubilized overnight at room temperature under gentle shaking in a 0.05 M NaNO<sub>3</sub> solution containing 0.02% NaN<sub>3</sub>. They were then filtered through a 0.45 µm membrane (Millipore, Millex, HV). A sample of 50 µl of the filtered solution was loaded on the HPSEC-Viscotek system. High-performance size exclusion chromatography (HPSEC) was performed at 25 °C in a PL aquagel-OH mixed 8 µm column (Polymer laboratories, 300 x 7.5 mm) equipped with a PL aquagel -OH 8 µm guard column (Polymer laboratories, 50 x 7.5 mm) eluted at 1 ml/min with 0.05 M NaNO<sub>3</sub> containing 0.02% NaN<sub>3</sub>. The column was mounted in series with an UV detector (SpectraSERIES UV100) and with parallel-coupled RI (ERC 7517A) and Viscotek (T-50A, Viscotek) detectors. Data acquisition was carried out using the Trisec (Viscotek) software.

Viscometry measurements were also made using an Ubbelohde capillary viscometer (0.46 mm) thermostated at 25 °C. Pectins (2 mg/ml) were solubilized overnight at room temperature under gentle shaking in a 0.05 M NaNO<sub>3</sub> solution containing 0.02% NaN<sub>3</sub>. They were then filtered through a 0.45 µm membrane (Millipore, Millex, HV). Intrinsic viscosity was calculated by extrapolating to C=0 the Huggins (1) and Kraemer (2) equations:

$$(1) \quad \eta_{\text{red}} = \eta_{\text{spe}} / C = [\eta] + \lambda_{\text{H}} C [\eta]^2$$

$$(2) \quad \eta_{\text{inh}} = \ln (\eta_r / C) = [\eta] - \lambda_{\text{K}} C [\eta]^2$$

with :

$\eta_r$ = related viscosity	C = concentration (g/ml)
$\eta_{\text{inh}}$ = inherent viscosity	$[\eta]$ = intrinsic viscosity (ml/g)
$\eta_{\text{red}}$ = reduced viscosity	$\lambda_{\text{H}}$ = Huggins coefficient
$\eta_{\text{spe}}$ = specific viscosity	$\lambda_{\text{K}}$ = Kraemer coefficient

### Microscopy

Thin sections (about 1 µm) of fruit tissues (flesh, carpels and skin) were cut by hand and coloured (10 min) with 0.02% acridine orange (CI 46005) in a 0.1 M phosphate buffer (pH 7). Images of the cell-walls were collected by a Zeiss LSM 410 confocal inverted microscope (Zeiss, Le Pecq, France) used in epi mode with a 40 x 1.2 water-immersion objective. A 488 nm argon ion laser was used to excite the dye. A

**Table 1.** Composition of dietary fibres (g/100 g dry matter) in chaenomeles fruits (Thomas *et al.* 2000).

Genotypes	AIS	TDF <sup>a</sup>	SDF <sup>a</sup>	IDF <sup>a</sup>	Dm <sup>b</sup>	Fibre mean <sup>c</sup>	Groups <sup>d</sup>
<i>C. japonica</i>							
NV1944	27.8	29.1	9.0	20.1	10.1	28.4	a
RG822	29.2	30.4	7.3	23.1	12.4	29.8	a
NV1410	29.4	30.9	8.5	22.4	8.8	30.2	a
NV1718	32.9	34.8	7.6	27.2	8.7	33.9	b
NV1597	31.9	36.7	9.1	27.6	10.8	34.3	bc
D3122	34.0	35.5	8.3	27.2	12.3	34.8	bc
NV1473	32.8	37.1	9.1	28.0	10.5	34.9	bc
NV19108	32.6	37.5	10.2	27.3	9.5	35.1	bc
NV152	33.8	37.0	10.4	26.5	10.5	35.4	bc
D694	35.9	35.9	6.5	29.4	10.9	35.8	bc
NV9392	35.0	37.3	8.6	28.6	11.9	36.1	c
NV1511	34.3	38.0	10.1	27.9	10.5	36.2	c
<i>C. speciosa</i>							
RG480	37.5	39.2	9.2	30.0	14.3	38.4	d
Mean	32.9	35.3	8.8	26.6	10.9	34.1	
SE of mean	0.8	0.9	0.3	0.8	0.4	0.8	

<sup>a</sup>Results were obtained from duplicates, SE < 2%, <sup>b</sup>g dry matter/100 g fresh matter, <sup>c</sup>mean of AIS + TDF,

<sup>d</sup>Fischer's multiple range test (protected least significant difference, PLSD)

Long Pass filter (LP 515) allowed collection of fluorescence emission higher than 515 nm. Attenuation was kept constant (30) during the sequential extraction of the pectins. It was increased to 100 during observation of the unstained flesh tissue and to 1000 during observation of the wax layer covering the skin. The same conditions of contrast and brightness were used for all observations. Sequences of 25 x-y optical sections were collected at increments of 1µm in the z-axis. Images were captured by the Carl Zeiss LSM software.

### Statistical analysis

A multiway analysis of variance (ANOVA) was performed on the data. For each significant factor, a multiple range test (Fischer's least significant difference (LSD) procedure) was used.

## RESULTS

### Dietary fibre

The total dietary fibre was extracted using two different methods: the Alcohol Insoluble Solid method, which provides a measure of alcohol insoluble solids (AIS), and the Association of Official Analytical Chemists method (AOAC), which results in measurements of soluble dietary fibre (SDF) and insoluble dietary fibre (IDF). The AIS value was then compared with the total dietary fibre (TDF) value, which was obtained by combining the values of SDF and IDF.

The amount and the nature of monomeric sugars in the constituent polysaccharides were also determined after total hydrolysis of the fibre fractions. Furthermore, the composition of the fibre from different parts of the fruit was studied.

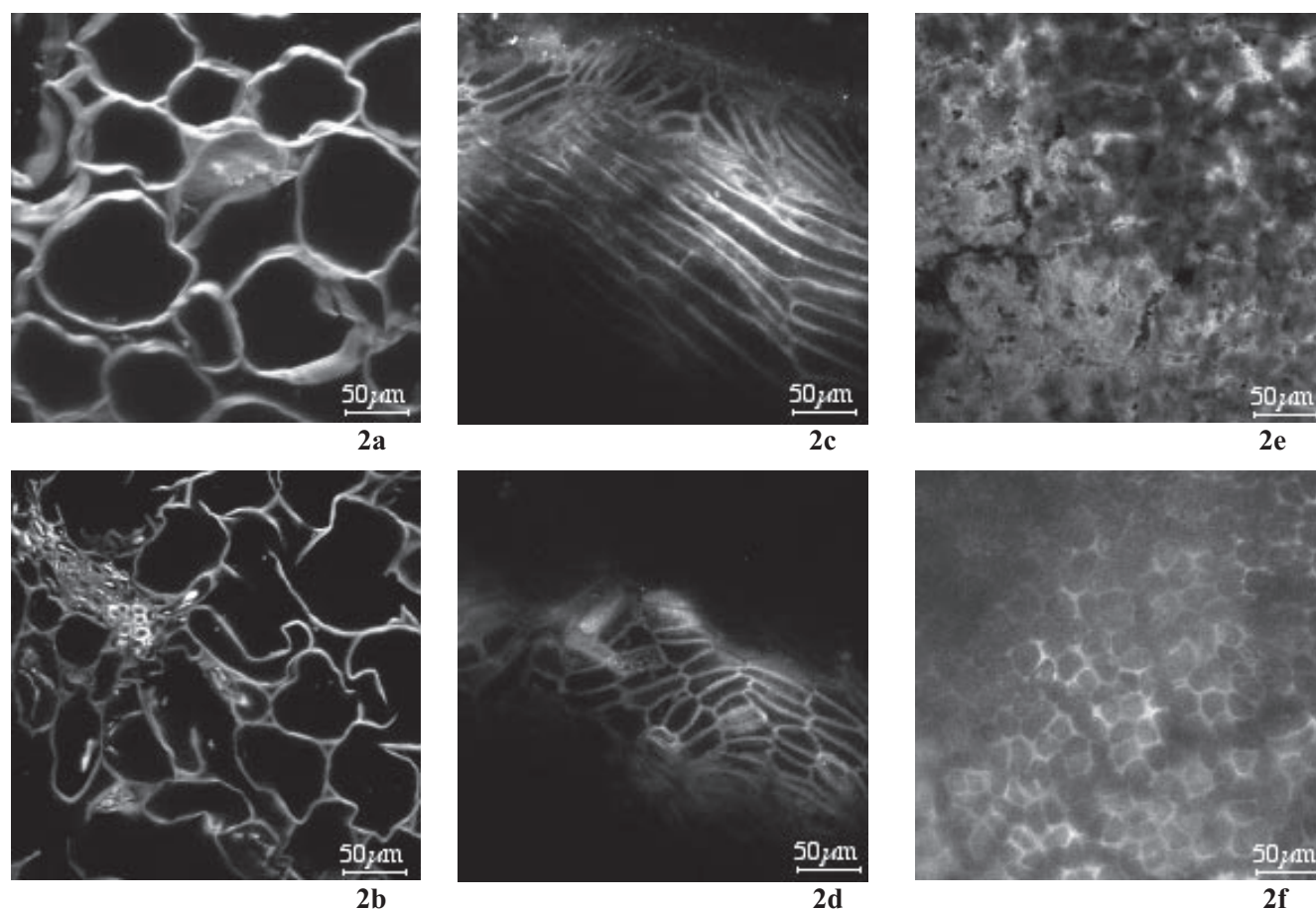
### Dietary fibre in the entire fruit

Table 1 summarises the content of AIS and dietary fibre in the fruits of the 13 genotypes. In the whole fruit, the AIS represented an average of 33 g/100 g dry matter and 3.6 g/100 g fresh matter. These results

are much higher than those obtained for apple by Massiot *et al.* (1994) and Renard *et al.* (1991), where the AIS represented only 12.6 g/100 g dry matter of the entire fruit and 2 g/100 g fresh weight of the flesh.

In the whole fruit, the TDF represented an average of 35 g/100 g dry matter (8.8 g SDF + 26.6 g IDF) and 3.9 g/100 g fresh fruit. This result is much higher than that obtained for apple by Renard *et al.* (1991) which was 1.7 g TDF/100 g fresh fruit.

The two methods for analysing dietary fibre (AIS and AOAC) resulted in significantly different estimates (Thomas *et al.* 2000). Fibre content was significantly higher when determined by the AOAC method compared to the AIS method, but there was no interaction between the samples and the methods (Thomas *et al.* 2000). Thus, it is possible to use any method to estimate dietary fibre and the ranking of genotypes should be consistent. Multiple range tests resulted in three homogeneous groups (Table 1). Group one, with a low content of fibre (NV1410, NV1944 and RG822); group two, with a moderate level of fibre (NV9392, D3122, D694, NV1473, NV1511, NV152, NV1597, NV1718 and NV19108); and group three with an isolated genotype (RG480, the only sample of *C. speciosa*).



**Figure 2.** Images of the different tissue-zones of the fruits observed with a CLSM (x40/1.2). Samples were stained with acridine orange.

2a) flesh, 25 focal planes, C 369, B9845, At 30, P 8.

2b) flesh, 1 focal plane, C 369, B9845, At 100, P 8.

2c) carpels, C 369, B9845, At 10, P 8.

2d) carpels, same focal plane as (c) (variation on the x-y axis), C 369, B9845, At 30, P 8.

2e) skin observed on the external surface, 1 focal plane, C 369, B9845, At 1000, P 8.

2f) same sample of skin as (e) observed on the internal surfaces, 1 focal plane, C 369, B9845, At 30, P 8.

C = contrast, B = brightness, At = attenuation, P = pinhole



### Dietary fibre in skin, flesh and carpels

The different tissue zones of genotype RG822 were observed in their initial state with a confocal laser scan microscope (Figure 2a–f). Figures 2a and 2b show a transverse section of the flesh of the fruit. The cells of the flesh have a spherical shape of about 50 µm diameter. The closer to the vessels, the smaller the cells (Figure 2b). Some cells have been broken by the freezing of the initial material or during the preparation of the thin layer. There are few intercellular spaces, their number probably depending on fruit maturity. Figures 2c and 2d show two images (distinct on the x–y axis) of the same focal plane of a longitudinal section of the carpels of the fruits. The cells of the carpel have an elongated shape. Their length varies from 90 to 180 µm and their width is about 18 µm. The sample was taken in the coalescing zone of the carpels. Therefore, two distinguishable orientations of the cells can be observed in the same focal plane (z axis). The cells of the carpels have a thick cell-wall and no intercellular spaces can be observed. Figures 2e and 2f show two views of the same sample of the fruit skin. The outer surface is covered by a wax layer, which has no specific organisation (Figure 2e). The cells of the skin can be seen on the inner surface of the skin (Figure 2f). They have a spherical shape of about 25 µm diameter and have a thick cell-wall. No intercellular spaces can be observed.

The flesh was the major fruit tissue, representing more than 70 g/100 g dry fruit. Skin and carpels comprised 7 and 23 g/100 g dry fruit, respectively (Table 2). The tissues of the fruit differed in their content of AIS and TDF. The flesh contained less cell-wall material (28 g/100 g dry flesh) than the skin and carpels (48 and 64 g/100 g dry zone, respectively). As the flesh represented 70 g/100 g dry fruit, most of the dietary fibre (52 g/100 g extracted fibre) was contained in the flesh. Experimental results obtained for the entire fruits of genotypes D694 and NV1511 were very close to those calculated using the distribution of weight for the different zones after dissection.

### Neutral and acidic sugars in the dietary fibre of the entire fruits

Content of total acidic sugars and neutral sugars was 76 g/100 g estimated as AIS and 78 g/100 g estimated as TDF (Tables 3 and 4). Other components could be proteins, substituents of the pectins (acetic acid, methanol) and non-sugar cell-wall constituents such as polyphenols or lignin. A comparison of non-cellulosic glucose in TDF and AIS showed that starch was at most a minor component.

For both materials (AIS and TDF), on average 27 g total sugars/100 g dry matter of the whole dry fruit was obtained. Acidic sugars represented on average 21 g/100 g AIS and 24 g/100 g TDF and neutral sugars 55 g/100 g AIS and 54 g/100 g TDF (Tables 3 and 4). These results were similar to those obtained for apple by Bittner *et al.* (1982) (acidic sugars: 18.7 g/100 g AIS, neutral sugars: 58.9 g/100 g the AIS).

**Table 2.** Distribution of weight, AIS and TDF in different tissue zones of two selected genotypes of Japanese quince (Thomas *et al.* 2000).

Genotype	D694					NV1511				
	Weight <sup>a</sup>	AIS <sup>a</sup>	TDF <sup>ab</sup>			Weight <sup>a</sup>	AIS <sup>a</sup>	TDF <sup>ab</sup>		
			IDF	SDF	TDF			IDF	SDF	TDF
Skin	7	3.2	3.3	0.5	3.8	6	2.4	2.6	0.5	3.1
Flesh	71	19.3	14.4	10.9	25.3	70	18.0	13.0	6.8	19.8
Carpels	22	13.5	10.6	3.1	13.7	24	14.8	13.2	3.9	17.1
Entire fruit (calculated)	100	36.0	28.3	14.5	42.8	100	35.2	28.8	11.2	40.0
Entire fruit (determined)	100	35.9	29.4	6.5	35.9	100	34.3	27.9	10.1	38.0

<sup>a</sup>g/100 g dry matter of the entire fruit, <sup>b</sup>results were obtained from duplicates, SE < 2%

**Table 3.** Content and composition of sugars and protein in **AIS** of chaenomeles fruits (Thomas *et al.* 2000).

Genotype	Yield <sup>a</sup>	Sugar composition <sup>bc</sup>										Prot. <sup>b</sup>
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc <sub>c</sub>	Glc <sub>nc</sub>	GalA	Tot.	
<i>C. japonica</i>												
NV1944	27.8	1.2	0.7	10.9	7.7	1.9	7.0	27.1	2.9	24.6	81.7	6.7
RG822	29.2	1.2	0.7	10.2	8.0	1.8	6.1	30.0	2.0	21.0	78.6	8.0
NV1410	29.4	1.0	0.6	6.1	5.4	2.1	4.9	26.8	1.8	23.5	67.5	7.3
NV1718	32.9	0.9	0.6	8.2	7.3	1.7	4.7	25.8	1.5	20.9	77.3	5.3
NV1597	31.9	1.1	0.7	9.4	9.3	1.8	5.8	25.6	3.2	23.1	79.8	5.7
D3122	34.0	1.0	0.6	12.0	9.5	1.8	7.9	27.4	2.3	19.1	72.8	5.1
NV1473	32.8	0.8	0.4	7.0	7.1	1.5	4.4	24.7	1.0	20.7	73.8	5.8
NV19108	32.6	0.8	0.5	7.3	8.6	1.6	4.0	27.1	1.4	21.2	83.9	6.2
NV152	33.8	0.8	0.6	9.4	5.9	1.9	7.2	24.9	1.5	21.6	72.3	5.5
D694	35.9	0.7	0.6	8.3	6.2	2.1	9.7	25.3	1.8	17.9	72.3	5.2
NV9392	35.0	0.8	0.5	8.7	8.3	2.0	7.4	26.7	1.8	18.5	74.6	4.7
NV1511	34.3	1.0	0.6	8.5	8.1	1.9	6.1	26.7	1.9	22.6	71.6	6.5
<i>C. speciosa</i>												
RG480	37.5	1.0	0.5	7.0	8.1	2.7	4.4	32.6	1.8	20.5	80.8	5.4
Mean	32.9	1.0	0.6	8.7	7.7	1.9	6.1	27	1.9	21.2	75.9	6.0
SE of mean	0.8	0.0	0.0	0.5	0.3	0.1	0.5	0.6	0.2	0.5	1.3	0.3

<sup>a</sup>g/100 g dry fruit, <sup>b</sup>g/100 g AIS, Glc<sub>c</sub> = cellulosic glucose, Glc<sub>nc</sub> = non cellulosic glucose, <sup>c</sup>results were obtained from duplicates, SE < 5%

**Table 4.** Content and composition of sugars and protein in **TDF** of chaenomeles fruits (Thomas *et al.* 2000).

Genotype	Yield <sup>a</sup>	Sugar composition <sup>bc</sup>										Prot. <sup>b</sup>
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc <sub>c</sub>	Glc <sub>nc</sub>	GalA	Tot.	
<i>C. japonica</i>												
NV1944	29.1	1.2	0.6	7.9	6.8	4.0	6.7	26.8	3.0	29.2	86.2	nd
RG822	30.4	1.2	0.6	6.7	7.8	4.1	4.7	28.4	2.5	23.9	79.9	nd
NV1410	30.9	1.1	0.6	5.9	6.8	4.3	5.8	25.9	2.9	26.8	80.2	nd
NV1718	34.8	0.9	0.4	3.0	11.4	2.6	2.6	25.4	1.8	23.6	71.7	nd
NV1597	36.7	1.0	0.5	6.2	12.2	3.5	4.6	26.4	2.4	23.5	80.2	nd
D3122	35.5	0.8	0.6	8.1	9.0	3.4	6.8	25.0	2.1	23.8	79.6	nd
NV1473	37.1	0.9	0.5	5.1	10.0	3.7	4.1	25.9	1.9	23.2	75.1	nd
NV19108	37.5	0.9	0.5	4.1	9.8	3.3	3.4	27.3	2.0	25.5	76.8	nd
NV152	37.0	0.9	0.6	7.0	7.2	4.0	6.5	24.1	2.8	26.5	79.6	nd
D694	35.9	0.9	0.6	6.0	9.2	3.9	7.4	28.0	3.3	19.6	79.0	nd
NV9392	37.3	0.8	0.5	6.3	9.2	3.4	7.0	26.1	2.2	20.2	75.6	nd
NV1511	38.0	0.9	0.5	5.3	8.1	3.7	5.6	24.3	2.5	23.8	74.8	nd
<i>C. speciosa</i>												
RG480	39.2	1.1	0.6	5.1	6.8	4.2	4.0	28.6	2.3	25.3	77.9	nd
Mean	35.3	1.0	0.5	5.9	8.8	3.7	5.3	26.3	2.4	24.2	78.2	nd
SE of mean	0.9	0.0	0.0	0.4	0.5	0.1	0.4	0.4	0.1	0.7	1.0	nd

<sup>a</sup>g/100 g dry fruit, <sup>b</sup>g/100 g TDF, Glc<sub>c</sub> = cellulosic glucose, Glc<sub>nc</sub> = non cellulosic glucose, <sup>c</sup>results were obtained from duplicates, SE < 5%

The total sugar content for IDF was 77 g/100 g and for SDF 83 g/100 g (Tables 5 and 6). In the IDF, the other constituents may be polyphenols and lignin, whereas the pectin substituents may be in the SDF. The total sugar content of the IDF was similar to that obtained for apple by Renard *et al.* (1991) (77 g/100 g IDF), whereas the total sugar content of the SDF, 83g/100 g, was much higher than that described for apple by Renard *et al.* (1991) (68 g/100 g SDF). The main component (Table 5) of the SDF was GalA (56 g/100 g SDF). SDF also contained an important amount of Ara (10 g/100 g SDF), Gal (4 g/100 g SDF), and Man (7 g/100 g SDF). These results are very close to the results obtained for apple by Renard *et al.* (1991) except for the Man content, which was higher in chaenomeles fruits. This implies that SDF was to a large extent composed of pectic substances. IDF was to a large extent contained glucose (38 g/100 g IDF) and xylose (11 g/100 g IDF). This implies that IDF was to a large extent composed of cellulose and hemicelluloses, such as xyloglucans (Table 6).

For further quantification of plant cell-walls the AIS method for measuring dietary fibre was selected. This was because oligosaccharides and other low molecular or alcohol-soluble products could be solubilized from the AIS sugars, whereas water-soluble polymers, such as pectins, remained in the residue. However, the AIS method involves coprecipitation of intracellular components such as proteins, nucleic acids, polyphenols and starch. The method may also give rise to artefacts due to collapse effects of the cell-walls when dehydrated by ethanol (Selvendran & O'Neill 1987). The AIS samples obtained are therefore difficult to hydrolyse and the amount of sugar analysed could be underestimated. However, measurement of SDF and IDF by the AOAC method may also give rise to artefacts since the method includes incubations in warm, neutral to alkaline conditions that can lead to degradation of pectins by b-elimination (Albersheim *et al.* 1960, Massiot *et al.* 1988).

#### Neutral and acidic sugars in the dietary fibre of the different tissue zones of the fruit

The sugar composition of the AIS was determined for each tissue zone of two genotypes of Japanese quince (Table 7). For the genotype D694, the carbohydrate content was 39, 92 and 74 g/100 g AIS of the

**Table 5.** Content and composition of sugars in SDF of chaenomeles fruits (Thomas *et al.* 2000).

Genotype	Yield <sup>a</sup>	Sugar composition <sup>b</sup>								
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc <sub>t</sub>	GalA	Tot.
<i>C. japonica</i>										
NV1944	9.0	1.5	0.4	14.2	1.0	7.7	5.8	2.4	62.2	95.1
RG822	7.3	2.1	0.4	10.7	1.2	8.6	4.9	2.0	57.9	87.9
NV1410	8.5	1.7	0.4	9.0	0.9	8.3	4.5	3.5	55.5	83.6
NV1718	7.6	1.4	0.3	4.6	0.6	4.9	2.5	1.6	51.7	67.6
NV1597	9.1	1.4	0.3	12.9	0.7	8.1	4.8	2.0	56.4	86.7
D3122	8.3	1.1	0.3	15.7	0.7	8.1	6.0	1.9	56.5	90.5
NV1473	9.1	1.3	0.3	8.2	0.6	8.3	3.5	1.7	58.1	81.8
NV19108	10.2	1.5	0.3	6.3	0.6	6.0	2.8	1.8	60.7	79.9
NV152	10.4	1.3	0.3	10.7	0.5	6.6	4.9	1.6	57.8	83.8
D694	6.5	1.1	0.3	10.9	0.6	7.8	4.9	2.1	47.7	75.3
NV9392	8.6	0.9	0.3	7.4	0.6	4.8	4.2	2.0	40.7	60.8
NV1511	10.1	1.1	0.3	7.7	0.6	7.7	4.4	1.6	56.1	79.6
<i>C. speciosa</i>										
RG480	9.2	2.2	0.3	9.9	1.1	8.0	4.7	1.5	71.6	99.4
Mean	8.8	1.4	0.3	9.9	0.7	7.3	4.5	2.0	56.4	82.5
SE of mean	1.2	0.4	0.0	3.2	0.2	1.3	1.0	0.5	7.3	10.5

<sup>a</sup>g/100 g dry fruit, <sup>b</sup>g/100 g SDF, Glc<sub>t</sub> = total glucose

**Table 6.** Content and composition of sugars in **IDF** of chaenomeles fruits.

Genotype	Yield <sup>a</sup>	Sugar composition <sup>b</sup>									
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc <sub>c</sub>	Glc <sub>nc</sub>	GalA	Tot.
<i>C. japonica</i>											
NV1944	20.1	1.0	0.8	5.0	9.4	2.4	7.1	38.7	3.3	14.5	82.2
RG822	23.1	1.0	0.6	5.4	9.9	2.7	4.7	37.3	2.7	13.2	77.4
NV1410	22.4	0.8	0.7	4.8	9.0	2.8	6.3	35.8	2.7	15.9	78.8
NV1718	27.2	0.7	0.4	2.6	14.4	2.0	2.6	32.5	1.9	15.8	72.8
NV1597	27.6	0.8	0.5	4.0	16.0	1.9	4.5	35.1	2.5	12.7	78.1
D3122	27.2	0.7	0.6	5.7	11.5	2.0	7.1	32.7	2.1	13.8	76.2
NV1473	28.0	0.7	0.5	4.1	13.0	2.2	4.3	34.4	2.0	11.8	73.0
NV19108	27.3	0.7	0.6	3.3	13.2	2.2	3.7	37.6	2.1	12.3	75.6
NV152	26.5	0.8	0.7	5.6	9.8	2.9	7.1	33.6	3.3	14.2	78.0
D694	29.4	0.9	0.7	5.0	11.1	3.0	8.0	34.3	3.6	13.3	79.8
NV9392	28.6	0.7	0.6	5.9	11.7	3.0	7.9	33.9	2.3	14.0	80.1
NV1511	27.9	0.8	0.6	4.4	10.8	2.2	6.1	33.2	2.9	12.1	73.1
<i>C. speciosa</i>											
RG480	30.0	0.7	0.6	3.6	8.6	3.0	3.8	37.4	2.5	11.0	71.2
Mean	26.6	0.8	0.6	4.6	11.4	2.5	5.6	35.1	2.6	13.4	76.6
SE of mean	2.9	0.1	0.1	1.0	2.2	0.4	1.8	2.1	0.5	1.5	3.3

<sup>a</sup>g/100 g dry fruit, <sup>b</sup>g/100 g IDF, Glc<sub>c</sub> = cellulosic glucose, Glc<sub>nc</sub> = non cellulosic glucose,

dry fruit zone in the skin, flesh and carpels, respectively. It was similar for the genotype NV1511 (50, 89 and 72 g/100 g AIS of the dry fruit zone). These results were very close to those obtained by Massiot & Renard (1997) for apple (skin: 47, flesh: 87 and carpels: 72 g/100 g AIS of the dry zone) and suggest that the content of cell-wall polysaccharides was higher in the flesh than in the skin. The low level of carbohydrates in the epidermis zone may be due to the presence of cutin-like substances (Massiot *et al.* 1994).

Analysis of the AIS indicated that the carbohydrate polymers were mostly pectic polysaccharides and glucans: GalA: 13–28 g/100 g, Ara: 5–12 g/100 g, Gal: 2–15 g/100 g, Glc: 12–31 g/100 g. Most of the GalA, 80%, was located in the flesh of the fruit whereas 15% was in the carpels and 5% in the skin of genotype D694. Results were similar for the genotype NV1511 (flesh 89%, carpels 8%, and skin 3% GalA). Moreover, Ara and Gal, the main non-cellulosic sugars, were also mainly located in the flesh in the fruits of genotype D694: Ara 83% and Gal 91%. Results were similar for the genotype NV1511: 81% and 90% for Ara and Gal, respectively. Almost 100% of the non-cellulosic glucose was also contained in the flesh. These results are in agreement with the distinction between SDF and IDF for each zone of the fruits (Table 2). Per 100 g SDF of the entire fruit, an average of 68 g was located in the flesh and was mostly composed of GalA, Ara and Gal (39, 13 and 5 g/100 g total sugars in SDF for D694 and 62, 8 and 5 g/100 g total sugars in SDF for NV1511).

AIS from the skin contained only 12.1 g Glc/100 g AIS (15.0 g for NV1511), whereas that from the flesh and the carpels contained about 30 g Glc/100 g AIS each. These results are in agreement with the distribution of soluble and insoluble fibre. Of the total extracted IDF, 78% was located in the carpels (Table 2). This insoluble fibre was composed of cellulosic glucose (43 g/100 g IDF for both D694 and NV1511) and xylose (25 and 24 g/100 g IDF for D694 and NV1511, respectively). Thus, in the carpel zone, glucose arose from insoluble polymers, cellulose and hemicellulosic polymers such as xyloglucans, which had also been found in apple (Renard *et al.* 1990).

**Table 7.** Content and composition of sugars and proteins in AIS of different tissue zones in Japanese quince fruits (*C. japonica*) genotypes D694 and NV1511.

Genotype	Yield <sup>a</sup>	Sugar composition <sup>b</sup>									Tot.	Prot. <sup>b</sup>
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc <sub>c</sub>	Glc <sub>nc</sub>	GalA		
D694												
Skin	3.5	0.5	0.3	6.0	1.7	1.0	3.0	12.1	0.0	14.0	38.6	11.3
Flesh	19.3	1.1	0.8	11.5	5.0	3.3	14.7	29.7	3.3	22.1	91.5	6.2
Carpels	13.5	0.6	0.0	5.6	18.7	1.2	3.6	30.8	0.0	13.0	73.5	2.8
Entire fruit <sup>c</sup>	36.0	0.9	0.5	8.8	9.8	2.3	9.5	28.5	1.8	18.0	80.0	5.4
NV1511												
Skin	2.4	0.9	0.4	7.9	2.0	1.2	3.4	15.0	0.0	19.2	50.0	11.5
Flesh	18.0	1.0	0.8	10.5	4.9	2.4	9.9	29.3	2.2	27.7	88.7	7.4
Carpels	14.8	0.7	0.2	5.4	17.4	1.2	2.3	25.4	3.6	15.4	71.6	3.4
Entire fruit <sup>c</sup>	35.2	0.9	0.5	8.2	10.0	1.8	6.3	26.7	2.6	21.9	78.9	6.0

<sup>a</sup>g/100 g dry fruit, <sup>b</sup>g/100 g AIS, Glc<sub>c</sub> = cellulosic glucose, Glc<sub>nc</sub> = non cellulosic glucose, <sup>c</sup>calculated

Protein values (5.2–6.5 g/100 g AIS and 5.2–4.3 g/100 g IDF of the entire fruit) were in the same range as those previously reported for apple by Massiot *et al.* (1994), Renard *et al.* (1991), Voragen *et al.* (1983) and Claye *et al.* (1996). The skin contained a higher proportion of proteins.

Ash was measured in each sample (results not shown). The amount of ash in the AIS and the IDF was very low (< 1%, except in the skin, where it represented about 1% of the AIS or IDF), whereas ash in SDF was about 9% due to the phosphate buffer used for enzymic degradation (Sungsoo *et al.* 1997).

### Cell-wall polysaccharides

In the sequential procedure that was used to extract pectic polysaccharides from the cell-walls (Figure 1), water solubilises pectins that are not strongly linked to the cell-wall. Potassium oxalate is presumed to solubilise pectins linked to the cell-wall by ionic interactions (Selvendran & O'Neill 1987). Dilute acid and dilute alkali solubilise pectins that are more strongly linked to the cell-wall (ester linkages, covalent linkages...). Hemicelluloses were extracted using a different sequential method, avoiding the acidic extraction step in order to minimize degradation of the hemicelluloses. Alkaline degradation (peeling) of hemicelluloses was also minimized by using sodium borohydride (Selvendran & O'Neill 1987). At each extraction step of the two sequences, the insoluble residues were not dried to avoid further irreversible collapse of cell-walls, which could hinder the subsequent extractions. Therefore, no intermediate yields could be calculated.

At each extraction step, the cells of the flesh were observed (using a confocal laser scan microscope) and compared with their initial state. A check was first made to ensure that no autofluorescence was present by observing unstained cells of the flesh. Fluorescence was thus a result of staining with acridine orange, a metachromatic dye employed as a fluorochrome to display the presence of anions (Conns 1990). In our study it was chosen as an indicator for the presence of pectins in the cell-wall.

### Cell-wall polysaccharides in the entire fruit

The results of the sequential extraction of the entire fruits and different tissue zones of genotypes RG822 and NV9392 are presented in Table 8. The sum of the weight of the extracted cell-wall polysaccharides and the final residues was > 80%, indicating that only a small amount was lost during the extractions, except (perhaps) for the flesh and the skin, for which recoveries were lower. This suggests that some

**Table 8.** Distribution of cell-wall polysaccharides extracted from different tissue zones in fruits of two selected genotypes of Japanese quince (*C. japonica*), RG822 and NV9392 (Thomas & Thibault 2002).

Genotype	Weight <sup>a</sup>	AIS <sup>b</sup>	Pectins <sup>c</sup> (Sequence A)					Hemicelluloses <sup>c</sup> (Sequence B)					
			P <sub>w</sub>	P <sub>O</sub>	P <sub>H</sub>	P <sub>OHa</sub>	ΣP	R <sub>p</sub>	P <sub>OHB</sub>	HC <sub>1</sub>	HC <sub>4</sub>	ΣP+HC	R <sub>HC</sub>
RG822													
Skin	7.9	45.7	7.7	1.9	19.9	8.2	37.7	42.0	21.7	5.3	4.1	40.7	54.0
Flesh	68.0	27.3	3.6	1.9	11.9	4.5	21.9	73.0	10.8	4.6	2.8	23.7	76.0
Carpels	24.1	51.4	2.5	1.5	14.6	6.3	24.9	71.0	7.5	3.8	3.6	18.9	58.0
Entire fruit <sup>d</sup>	100.0	34.6	5.7	1.8	16.5	6.7	30.7	56.1	16.3	4.9	3.6	32.3	62.3
NV9392													
Skin	9.2	48.0	7.4	1.9	20.1	8.1	37.5	51.0	14.8	5.8	4.7	34.6	62.0
Flesh	66.0	30.2	3.0	1.5	10.6	2.8	17.9	77.0	6.8	3.8	1.4	16.5	81.0
Carpels	24.8	61.6	3.0	2.2	18.3	3.6	27.1	69.0	10.2	3.8	3.1	22.3	43.0
Entire fruit <sup>d</sup>	100.0	39.6	5.2	1.8	16.2	5.6	28.8	63.1	11.2	4.8	3.3	26.3	67.2

<sup>a</sup>g/100 g dry fruit, <sup>b</sup>g/100 g tissue zone, <sup>c</sup>g/100 g AIS, <sup>d</sup>calculated

oligosaccharides or some non-carbohydrate material may have been lost during dialysis of the extracts or during the successive extractions.

The pectins (P<sub>w</sub>+P<sub>O</sub>+P<sub>H</sub>+P<sub>OHa</sub>) represented 31 and 29 g/100 g AIS for genotypes RG822 and NV9392, respectively. Water solubilized 18.6 and 18.1% of the total extracted pectins for genotypes RG822 and NV9392, respectively and potassium oxalate 5.9 and 6.3%. Pectins weakly linked to the cell-wall represented less than 25% of the total extracted pectins, whereas pectins solubilized by dilute acid represented 53.7 and 56.3% of the total extracted pectins for genotypes RG822 and NV9392, respectively. An additional quantity of pectins could be extracted by a dilute alkali (21.8 and 19.4% of total extracted pectins for genotypes RG822 and NV9392, respectively).

The quantities of pectins extracted by water and oxalate were equivalent in sequences A and B, and were lower than pectins extracted by hot dilute acid. Moreover, the total quantity of pectins extracted by 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<sub>w</sub>+P<sub>O</sub>+P<sub>H</sub>+P<sub>OHa</sub> = 38 g/100 g AIS of the flesh for both RG822 and NV9392 and P<sub>w</sub>+P<sub>O</sub>+P<sub>OHB</sub> = 31 and 24 g/100 g AIS of the flesh of RG822 and NV9392, respectively. Thus, pectins were more efficiently extracted when a step including hot dilute acid was used in the extraction scheme. This result is in agreement with studies on beets (Rombouts & Thibault 1986) and grapes (Saulnier & Thibault 1987), but disagrees with results obtained for carrots (Massiot *et al.* 1988) and apples (Massiot *et al.* 1994).

The hemicelluloses represented a very small proportion of total extracted polysaccharides: HC<sub>1</sub>+HC<sub>4</sub> = 8.5 and 8.1 g/100 g AIS of the entire fruits of the genotypes RG822 and NV9392, respectively. No precipitation of the hemicelluloses occurred during neutralisation of HC<sub>1</sub> and HC<sub>4</sub> with HCl, and it was possible to extract slightly higher quantities of hemicelluloses with 1M KOH than with 4M KOH.

The cellulosic residues represented more than 56 g/100 g AIS. No significant difference in content of pectins or hemicelluloses was observed between the two genotypes studied, but NV9392 had a higher content of cellulosic residue than RG822 (R<sub>p</sub> = 56 and 63 g/100 g AIS, and R<sub>HC</sub> = 62 and 67 g/100 g AIS for RG822 and NV9392, respectively).

### Cell-wall polysaccharides in the different tissue zones

The cells in the fruit flesh had a distorted shape after preparation of AIS (Figure 3a), probably due to the 20 min boiling in ethanol. The quantity of intercellular spaces had, however, not increased, and the

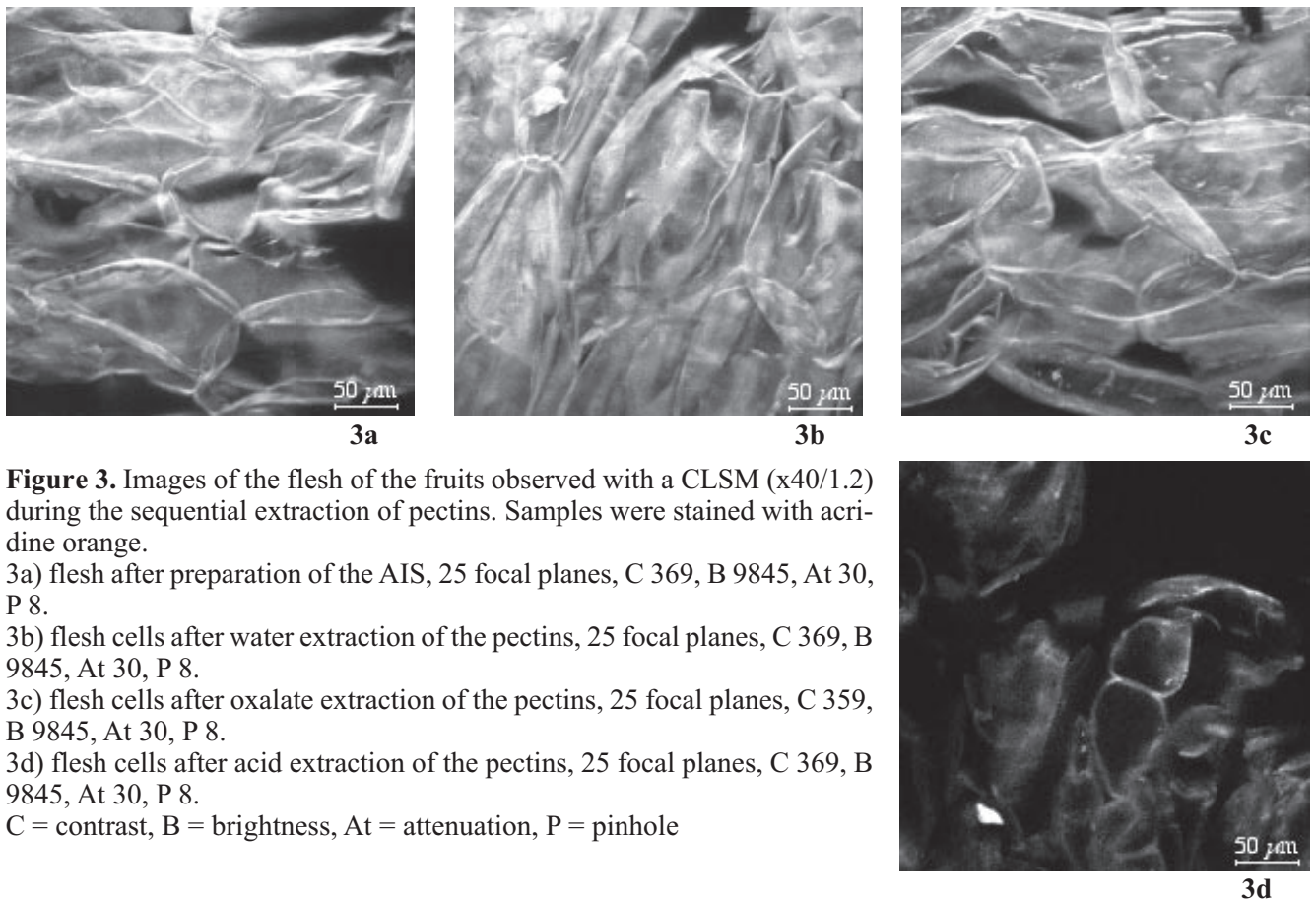
fluorescence was strong. After water or oxalate extraction, the topology of the tissue did not change (Figure 3b and c). The cells had a distorted shape, the number of intercellular spaces had not increased and fluorescence was strong, indicating that pectins were still present in the residue.

After the acidic extraction (Figure 3d), the topology of the tissue was completely changed. The cells were separated and the fluorescence had decreased dramatically, indicating that pectins had been removed from the cell-wall.

### Pectins and hemicelluloses in different tissue zones of the fruit

On average, 66% of the extracted pectins came from the flesh, 10% from the skin and 24% from the carpels (Table 8). For genotype RG822,  $P_w + P_o + P_h + P_{OHa}$  was 37.7, 20.9 and 24.9 g/100 g AIS in the flesh, carpels and skin, respectively. The cellulosic residue represented more than 70 g/100 g AIS in the skin and the carpels. The amounts of pectins extracted from the skin, flesh or carpels differed slightly, depending on the extraction medium used. On average, 11, 7, 63 and 19% pectins were solubilised from the skin, whereas 20, 5, 53 and 22% pectins were solubilised from the flesh, and 15, 9, 58 and 19% pectins were solubilised from the carpels, with water, oxalate, HCl and KOH, respectively. The results were similar for genotype NV9392.

The flesh contained higher quantities of hemicelluloses (9.4 and 10.5 g/100 g AIS of the flesh for genotypes RG822 and NV9392, respectively) than the carpels (7.4 and 5.2 g/100 g AIS of the carpels for genotypes RG822 and NV9392, respectively) or the skin (7.4 and 6.9 g/100 g AIS of the flesh for genotypes RG822 and NV9392, respectively). Cellulosic residues represented the main part of the AIS. The quantities of cellulosic residue were higher in the skin (71 and 69 g  $R_p$ /100 g AIS of the skin for genotypes RG822 and NV9392, respectively) and the carpels (73 and 77g  $R_p$ /100 g AIS of the carpels for



**Figure 3.** Images of the flesh of the fruits observed with a CLSM (x40/1.2) during the sequential extraction of pectins. Samples were stained with acridine orange.

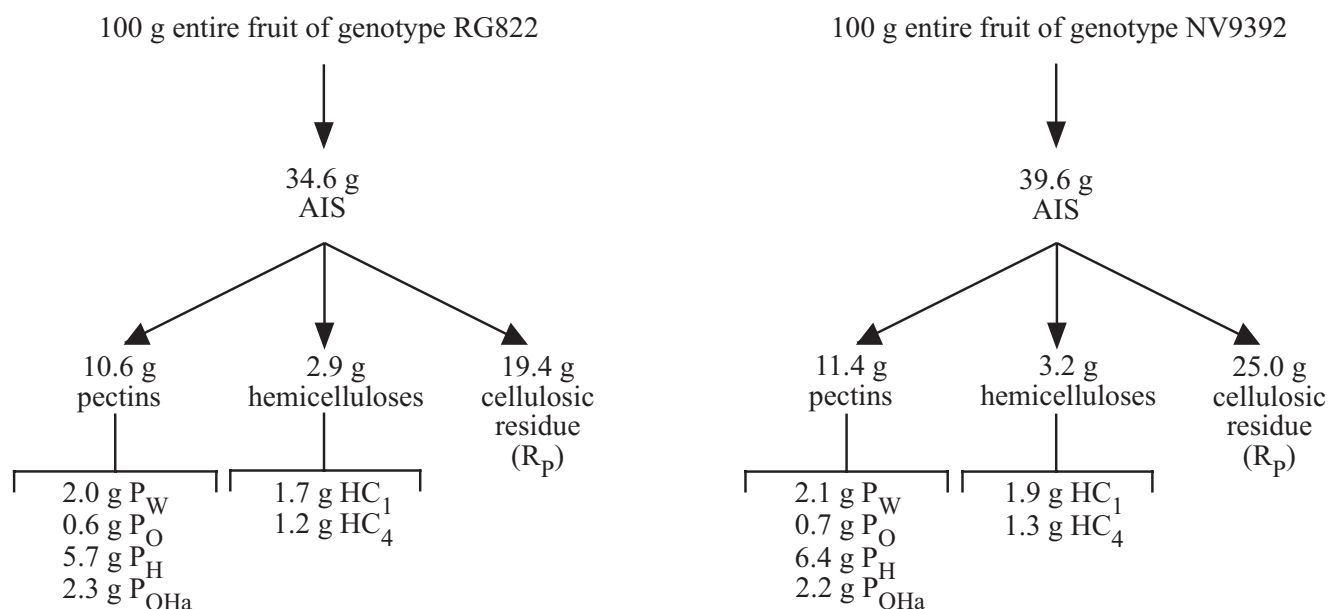
3a) flesh after preparation of the AIS, 25 focal planes, C 369, B 9845, At 30, P 8.

3b) flesh cells after water extraction of the pectins, 25 focal planes, C 369, B 9845, At 30, P 8.

3c) flesh cells after oxalate extraction of the pectins, 25 focal planes, C 359, B 9845, At 30, P 8.

3d) flesh cells after acid extraction of the pectins, 25 focal planes, C 369, B 9845, At 30, P 8.

C = contrast, B = brightness, At = attenuation, P = pinhole



**Figure 4.** Content of cell-wall polysaccharides (on dry matter basis) in the two selected genotypes of Japanese quince (*C. japonica*), RG822 and NV9392.

genotypes RG822 and NV9392, respectively) than in the flesh of the fruit (42 and 51 g R<sub>p</sub>/100 g AIS of the flesh for genotypes RG822 and NV9392, respectively). The calculated and experimental results were in rather good agreement.

### Cell-wall polysaccharides in Japanese quince fruits

A summary of the different quantities of polysaccharides in the fruits and tissues of the two Japanese quince genotypes RG822 and NV9392 is shown in Figure 4. On average, it was possible to extract 11.0 g pectins and 3.1 g hemicelluloses from 100 g dry fruit, and 22.2 g of cellulosic residues were obtained. Pectins and hemicelluloses came essentially from the flesh of the fruit (65.7 and 62.5%, respectively), whereas the cellulose was mainly located in the carpels (46.9%) and in the flesh (40.4%).

### Composition of the pectins

The composition of the P<sub>w</sub>, P<sub>o</sub>, P<sub>H</sub>, P<sub>OHa</sub> and P<sub>OHa</sub> is given in Table 9. In every extract from each tissue zone of the two genotypes RG822 and NV9392, UA, Rha, Ara and Gal were the main sugars detected, indicating that pectins were the main polysaccharides in these 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 on average 70 g/100 g extract. Other constituents may be substituents of the pectins (methanol, acetic acid), proteins and others. Pectins extracted from the carpels seemed to contain fewer 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 content of protein in the AIS of the skin (Table 7).

Independent of origin (tissue zone), hot dilute acid solubilised material rich in Ara and Gal. Approximately 80% and 60% of the Ara and of the Gal, respectively, were solubilised in this step. This suggests that these pectins (P<sub>H</sub>) have more or longer side-chains than the others (P<sub>w</sub>, P<sub>o</sub>, P<sub>OHa</sub>), which is in agreement with results reported by Massiot *et al.* (1994). It has been claimed (Selvendran *et al.* 1985) that P<sub>o</sub> may originate from the middle lamella, whereas P<sub>H</sub> comes from the primary cell-wall. In every extract of the various tissue zones of the two genotypes studied here, the molar ratio between uronic acids and rhamnose (UA/Rha) was not very high (44 on average), indicating that pectins had a low proportion of



**Table 9.** Composition of sugars, and degrees of methylation (DM) and acetylation (DAc) of pectins extracted from different tissue zones of two selected genotypes of Japanese quince (*C. japonica*), RG822 and NV9392.

Zone	Extract	Yield <sup>a</sup>	Sugar composition <sup>bc</sup>								DM	DAc
			Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA <sup>d</sup>		
RG822												
Skin	P <sub>W</sub>	2.5	0.7	0.3	9.2	1.5	0.7	5.0	3.5	54.1	73	5
	P <sub>O</sub>	1.5	0.6	0.0	5.7	0.5	0.0	2.0	0.0	45.0	68	6
	P <sub>H</sub>	14.6	1.1	0.2	19.4	0.7	0.1	5.6	1.7	38.3	73	7
	P <sub>OHa</sub>	6.3	1.2	0.2	3.4	1.0	0.0	4.3	1.2	46.7	4	2
	P <sub>OHb</sub>	7.5	0.8	0.0	5.9	0.5	0.0	2.4	0.4	54.6	8	3
Flesh	P <sub>W</sub>	7.7	0.9	0.2	7.6	0.9	1.4	4.7	3.0	49.0	71	3
	P <sub>O</sub>	1.9	1.0	0.1	6.7	0.8	0.2	3.7	2.3	43.2	64	3
	P <sub>H</sub>	19.9	1.2	0.2	12.6	1.0	0.3	6.3	1.3	54.8	61	3
	P <sub>OHa</sub>	8.2	2.1	0.3	3.9	1.9	0.0	8.1	1.2	48.2	7	1
	P <sub>OHb</sub>	21.7	1.3	0.2	7.7	0.6	0.0	5.5	0.4	52.2	7	1
Carpels	P <sub>W</sub>	2.6	0.8	0.2	8.4	1.1	1.6	4.2	2.8	61.5	70	7
	P <sub>O</sub>	1.9	0.7	0.0	4.9	0.5	0.5	2.2	1.1	58.0	60	4
	P <sub>H</sub>	11.9	1.4	0.2	15.9	0.7	0.0	5.4	1.1	48.4	60	8
	P <sub>OHa</sub>	4.5	1.8	0.2	3.1	1.6	0.0	6.5	0.8	56.3	4	3
	P <sub>OHb</sub>	10.8	1.2	0.1	6.9	0.3	0.0	3.0	0.1	60.7	5	3
NV9392												
Skin	P <sub>W</sub>	3.0	0.5	0.6	7.8	3.9	0.8	4.6	8.6	43.6	73	6
	P <sub>O</sub>	2.2	0.6	0.2	6.1	1.3	0.5	3.1	2.6	58.0	63	4
	P <sub>H</sub>	18.3	1.3	0.2	14.8	0.7	0.0	6.0	2.2	42.0	60	5
	P <sub>OHa</sub>	3.6	1.6	0.2	3.5	1.4	0.0	6.2	1.7	40.1	7	2
	P <sub>OHb</sub>	10.2	0.7	0.2	7.5	0.6	0.0	3.2	1.0	51.8	7	3
Flesh	P <sub>W</sub>	7.4	0.8	0.3	10.5	1.7	2.0	7.9	4.7	44.7	80	5
	P <sub>O</sub>	1.9	0.7	0.2	5.0	0.8	0.8	4.6	2.1	54.2	65	3
	P <sub>H</sub>	20.1	0.9	0.2	16.4	0.7	0.4	9.4	1.5	43.4	59	4
	P <sub>OHa</sub>	8.1	1.6	0.3	5.5	0.8	0.0	13.6	0.7	55.4	4	1
	P <sub>OHb</sub>	14.8	1.0	0.0	6.8	0.1	0.0	5.8	0.0	55.7	< 1	1
Carpels	P <sub>W</sub>	3.0	0.6	0.2	8.7	1.6	2.3	5.7	3.6	59.6	74	9
	P <sub>O</sub>	1.5	0.5	0.0	3.5	0.4	0.2	2.2	0.9	66.0	62	5
	P <sub>H</sub>	10.6	1.3	0.2	17.9	0.4	0.0	7.2	1.4	51.4	57	8
	P <sub>OHa</sub>	2.8	2.0	0.2	2.7	1.1	0.0	8.2	0.8	50.7	4	3
	P <sub>OHb</sub>	6.8	0.9	0.0	5.7	0.3	0.0	3.4	0.0	68.5	5	3

<sup>a</sup>g/100 g AIS, <sup>b</sup>g/100 g extract, <sup>c</sup>results were obtained from duplicates, SE < 5%, <sup>d</sup>UA = uronic acids, GlcA < 5%

regions carrying side-chains. These ratios decreased from oxalate- or water-soluble pectins to dilute-acid- or dilute-alkali-soluble pectins (P<sub>OHa</sub>). This result is in agreement with those found for apple by Renard *et al.* (1990). It suggests that dilute-acid-soluble pectins had a higher proportion of rhamnogalacturonans than water- or oxalate-soluble pectins, but a lower proportion of rhamnogalacturonans than dilute alkali-soluble pectins (P<sub>OHa</sub>). 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 molar ratio between uronic acids and rhamnose was the lowest for  $P_{\text{OHa}}$ , independent of origin, whereas it was intermediate for  $P_{\text{OHb}}$ . Sorting the molar ratios, the following chain was obtained:  $P_{\text{OHa}} < P_{\text{H}} < P_{\text{OHb}} < P_{\text{w}} < P_{\text{O}}$ , suggesting that pectins extracted with dilute alkali after an acidic treatment ( $P_{\text{OHa}}$ ) had a higher proportion of rhamnogalacturonans than those extracted with dilute alkali alone ( $P_{\text{OHb}}$ ). Dilute acid may have degraded side-chains, thus giving dilute alkali the possibility to liberate pectins with short side chains. Without acidic treatment, only less branched pectins can be liberated. The yield of pectins extracted with dilute alkali alone ( $P_{\text{OHb}}$ ) was approximately half that obtained with hot dilute acid followed by a dilute alkali extraction ( $P_{\text{H}} + P_{\text{OHa}}$ ). Moreover, alkali extraction following acidic extraction yielded a rather low amount of polysaccharides (from 2.8 to 8.2 g/100 g AIS). These results indicate that acid-labile linkages seemed to play a major role for the extraction of pectins. More Ara was extracted by dilute alkali alone ( $P_{\text{OHb}}$ ) than by dilute alkali following an acidic extraction ( $P_{\text{OHa}}$ ). This was probably due to the acidic hydrolysis of arabinose moieties into small oligomers. By contrast, Xyl and Gal were more efficiently extracted in  $P_{\text{OHa}}$  than in  $P_{\text{OHb}}$ .

Pectins from the flesh, carpels and skin seemed to have similar proportions of rhamnogalacturonans, as suggested by the constancy of the molar ratios between uronic acids and rhamnose. Pectins from the carpels had a slightly higher proportion of GalA compared to pectins 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 the skin had a similar GalA content.

Tables 8 and 9 show that the AIS of entire fruits of genotypes RG822 and NV9392 not only contained the same amount of pectins ( $P_{\text{w}} + P_{\text{O}} + P_{\text{H}} + P_{\text{OHa}}$  was 31 and 29 g/100 g AIS for RG822 and NV9392, respectively), but also that the composition of these pectins was very similar (71 and 68 g sugars/100 g extract for RG822 and NV9392, respectively). Thus, the difference observed in dietary fibre content of the two genotypes could not be attributed to the pectins.

The degrees of methylation and acetylation of pectins are given in Table 9. The degree of acetylation was calculated assuming only pectins were acetylated. Pectins had a high DM (range: 59–80) and a low DAc (range: 1–9). As anticipated, pectins extracted with alkali had low DM (< 8) and low DAc (< 3). There was no difference between the two genotypes studied in these traits.

### Composition of the hemicelluloses

The composition of the different extracts of hemicelluloses is given in Table 10. Some glucuronic acid has been found in some hemicelluloses (Selvendran & O'Neill 1987). In the hemicelluloses extracted from Japanese quince, less than 5 g GlcA/100 g UA was detected. The main constituent sugars of the extracts were Xyl, Man, Gal and Glc. These sugars are normal representatives of hemicellulosic fractions. The total acidic sugars represented less than 5 g/100 g extract. On average, total neutral sugars represented more than 50 g/100 g extracts except in the skin, where they represented only 46 g/100 g extract. Some non-carbohydrate constituents, such as proteins or polyphenols, may also have been present in the extracts of the skin.

Hemicelluloses extracted with 1 M KOH and those extracted with 4 M KOH differed in composition of monosaccharides.  $\text{HC}_1$  contained high proportions of Xyl, probably deriving from xylans, whereas  $\text{HC}_4$  contained high proportions of Man, probably deriving from mannans. These results were similar to those obtained for the cauliflower by Femenia *et al.* (1999).

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, and hemicelluloses from the flesh contained more Glc than hemicelluloses from the carpels and skin. No difference in the composition and quantity of hemicelluloses was observed between the two genotypes studied.

**Table 10.** Composition of sugars of hemicelluloses extracted from different tissue zones of two selected genotypes of Japanese quince (*C. japonica*), RG822 and NV9392.

Zone	Extract	Yield <sup>a</sup>	Sugar composition <sup>bc</sup>							UA <sup>d</sup>
			Rha	Fuc	Ara	Xyl	Man	Gal	Glc	
RG822										
Skin	HC <sub>1</sub>	3.8	0.7	1.6	6.1	12.4	3.2	7.1	14.9	3.2
	HC <sub>4</sub>	3.6	0.6	0.9	4.4	4.7	4.5	5.3	9.3	2.7
Flesh	HC <sub>1</sub>	5.3	0.5	3.3	4.2	22.0	2.0	10.7	29.3	2.6
	HC <sub>4</sub>	4.1	1.0	1.7	4.5	9.9	13.7	10.8	22.5	3.5
Carpels	HC <sub>1</sub>	4.6	0.6	1.7	3.2	36.0	2.9	6.8	16.2	5.4
	HC <sub>4</sub>	2.8	0.6	1.7	2.7	16.8	9.9	8.4	20.1	2.5
NV9392										
Skin	HC <sub>1</sub>	3.8	0.5	1.9	4.2	16.3	4.5	8.5	20.6	2.3
	HC <sub>4</sub>	3.1	0.6	0.6	4.8	6.9	7.0	6.9	13.2	3.2
Flesh	HC <sub>1</sub>	5.8	0.4	3.1	4.2	25.6	3.0	11.1	29.5	2.7
	HC <sub>4</sub>	4.7	0.8	1.6	4.5	10.0	12.8	11.1	21.3	2.3
Carpels	HC <sub>1</sub>	3.8	0.5	1.7	2.8	32.0	3.5	6.6	17.1	4.8
	HC <sub>4</sub>	1.4	0.6	1.8	2.8	24.0	6.1	7.5	17.8	3.7

<sup>a</sup>g/100 g AIS, <sup>b</sup>g/100 g extract, <sup>c</sup>results were obtained from duplicates, SE < 5%, <sup>d</sup>UA = uronic acids, GlcA < 5%

### Cellulosic residues

The composition of monosaccharides in the cellulosic residues is given in Table 11. Residues mostly consisted of neutral sugars, particularly Glc. Total neutral and acidic sugars represented only 34 and 55 g/100 g of R<sub>p</sub> and R<sub>HC</sub>, respectively, in the skin of RG822, and 26 and 53 g/100 g of R<sub>p</sub> and R<sub>HC</sub>, respectively, in 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) remained in the residues, showing that pectins were still present in the final residues. The composition of R<sub>p</sub> was very close to that of R<sub>HC</sub>. However, R<sub>HC</sub> contained more uronic acids (12 g UA/100 g extract, on average) than R<sub>p</sub> (6 g UA/100 g extract, on average) underlining the fact that the acidic extraction step was the most efficient in extracting pectins.

There was no major difference between the cellulosic residues from the different tissue zones of the fruit. However, the same differences observed for the hemicelluloses were also observed for the cellulosic residues. Thus, cellulosic residues from the carpels contained more Xyl than those from the flesh and the skin, and cellulosic residues from the flesh contained more Glc than cellulosic residues from the carpels and skin. This indicates that some hemicelluloses may be linked to the cellulose.

The different tissue zones of genotype NV9392 contained higher amounts of cellulosic residues than the different tissue zones of genotype RG822. However, residues from the different tissue zones of genotype NV9392 had a lower content of polysaccharides compared to the residues from the different tissue zones of genotype RG822. The difference in dietary fibre content of the two genotypes was thus not due to their constituent polysaccharides.

### Physico-chemical properties of pectins extracted from entire fruits of Japanese quince

The composition of pectins extracted by water, oxalate and dilute acid from entire frozen fruits was similar to what has previously been described for the fractionated pectins in different tissues. The physico-chemical properties of the P<sub>w</sub>, P<sub>O</sub> and P<sub>H</sub> were studied using the HPSEC-Viscotek system. The recovery of the polysaccharides after filtration was higher than 95% (results not shown), indicating that no

**Table 11.** Composition of sugars of cellulosic residues from different tissue zones of two selected genotypes of Japanese quince (*C. japonica*), RG822 and NV9392 (Thomas & Thibault 2002).

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

<sup>a</sup>g/100 g extract, <sup>b</sup>results were obtained from duplicates, SE < 5%, <sup>c</sup>UA = uronic acids, GlcA < 5%

polysaccharides were lost during filtration. Refractometric, viscosimetric and UV profiles of the P<sub>w</sub>, P<sub>o</sub> and P<sub>H</sub> are shown in Figures 5a to 5c for genotype RG822 and in Figures 5d to 5f for genotype NV9392. The UV profiles showed that there were no UV-absorbing substances in the P<sub>w</sub> and P<sub>o</sub>.

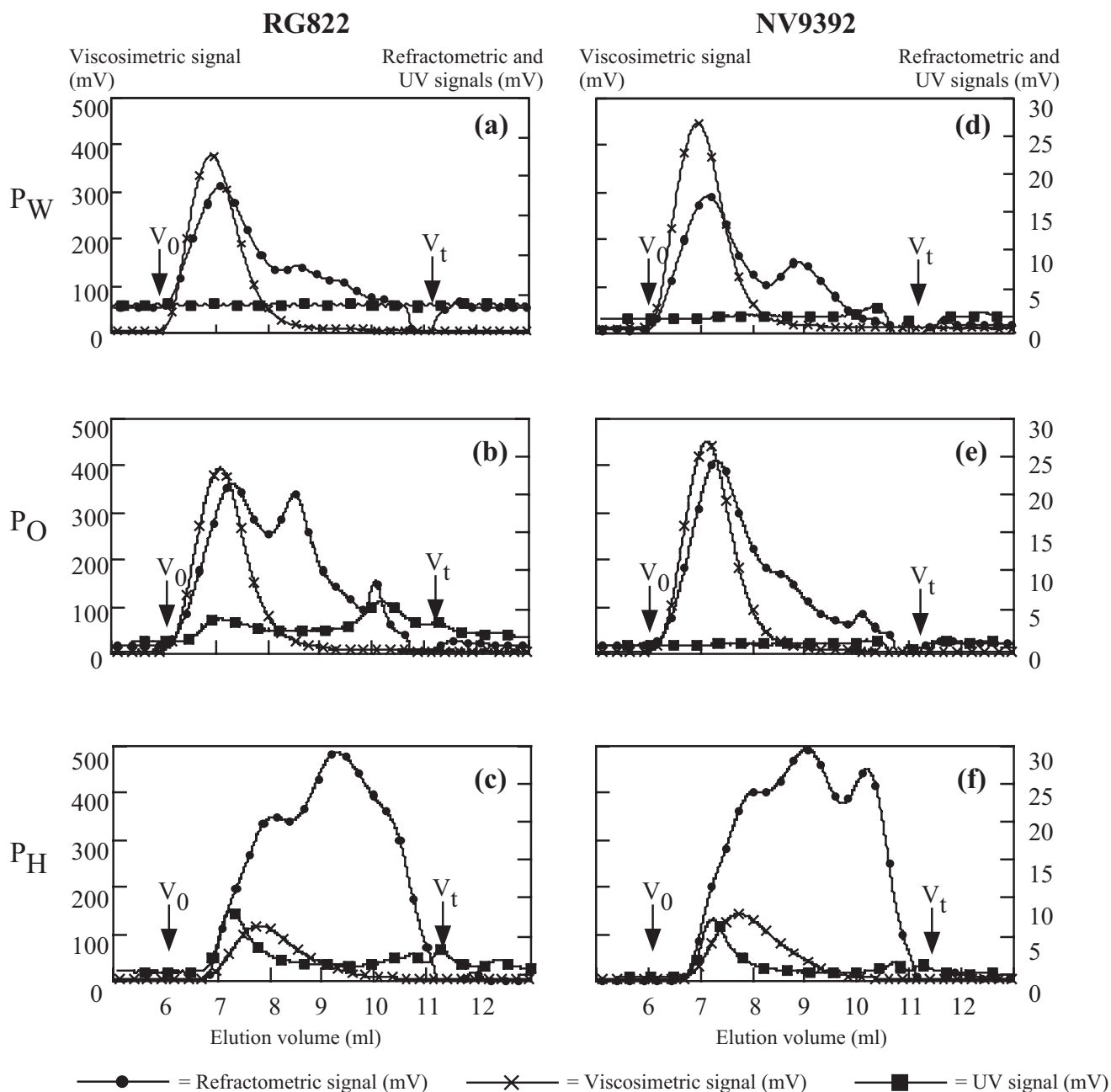
### Physico-chemical properties of P<sub>w</sub>

The chromatographic profiles showed that the P<sub>w</sub> consisted of at least two populations (Figures 5a and 5d). The first of these was eluted between 5.8 and 8.2 ml. It represented 25 and 32% of the total injected polysaccharides for genotypes RG822 and NV9392, respectively. The intrinsic viscosity of this population was 1399 and 1446 ml/g for genotypes RG822 and NV9392, respectively. The second constituent population of the P<sub>w</sub> was eluted between 8.2 and 10.1 ml. It represented 8 and 15% of the total injected polysaccharides for genotypes RG822 and NV9392, respectively. The intrinsic viscosity of this population was 106 and 72 ml/g for genotypes RG822 and NV9392, respectively. The intrinsic viscosity of the whole population of the P<sub>w</sub> was also determined. It was 1109 and 1071 ml/g for genotypes RG822 and NV9392, respectively. These results are very high compared to intrinsic viscosity estimates of acid-extracted apple pectins, which are usually between 250 and 470 ml/g (Cros *et al.* 1996, Axelos & Thibault 1991). Moreover, the chromatographic yields were low (32 and 47% of the total injected polysaccharides for genotypes RG822 and NV9392, respectively). Some polysaccharides may thus have remained bound to the column. Therefore, intrinsic viscosity of the P<sub>w</sub> was also measured with an Ubbelohde capillary viscometer (Figures 6a and 6c). Results (Table 12) showed that the intrinsic viscosity (804 and 647 ml/g for genotypes RG822 and NV9392, respectively) was still higher than those described in the literature for acid-extracted apple pectins.

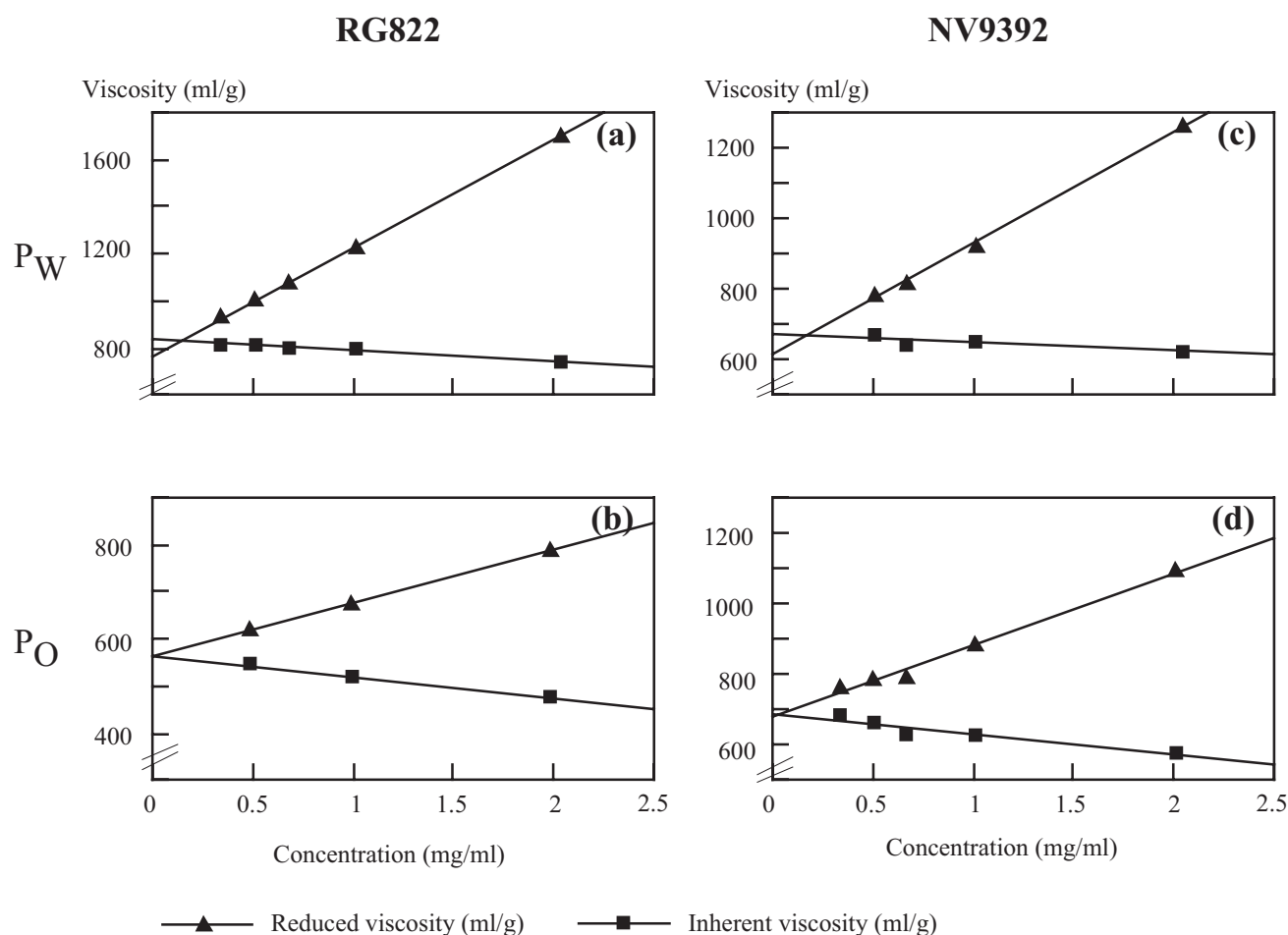
### Physico-chemical properties of P<sub>o</sub>

The chromatographic profiles showed that the P<sub>o</sub> consisted of three populations (Figures 5b and 5e). The first of these was eluted between 5.8 and 8.2 ml. It represented 32% of the total injected polysaccharides for both genotypes RG822 and NV9392. The intrinsic viscosity of this population was 1141 and 1104 ml/g for genotypes RG822 and NV9392, respectively. The second population of the P<sub>o</sub> was eluted between

8.2 and 10.1 ml. It represented 26 and 10% of the total injected polysaccharides for genotypes RG822 and NV9392, respectively. The intrinsic viscosity of this population was 76 and 113 ml/g for genotypes RG822 and NV9392, respectively. The intrinsic viscosity of the whole population of the  $P_o$  was also determined. It was 641 and 840 ml/g for genotypes RG822 and NV9392, respectively. These results were lower than those obtained for the  $P_w$ , but they were still very high compared to those described in literature for acid-extracted apple pectins (Cros *et al.* 1996, Axelos & Thibault 1991). As for the  $P_w$ , the chromatographic yields were low (58 and 42% of the total injected polysaccharides for genotypes RG822 and NV9392, respectively). Some polysaccharides may thus have remained bound to the column. Therefore, intrinsic viscosity of the  $P_o$  was also measured with an Ubbelohde capillary viscometer (Figures 6b and 6d). Results (Table 12) showed that intrinsic viscosity was still very high (564 and 687 ml/g for



**Figure 5.** Viscosimetric, refractometric and UV profiles of pectins,  $P_w$  (a and d),  $P_o$  (b and e) and  $P_H$  (c and f) extracted from entire frozen fruits of genotypes RG822 (a to c) and NV9392 (d to f).



**Figure 6.** Intrinsic viscosity of  $P_W$  (a and c) and  $P_O$  (b and d) for genotypes RG822 (a and b) and NV9392 (c and d).

genotypes RG822 and NV9392, respectively).

### Physico-chemical properties of $P_H$

The UV profiles of the  $P_H$  (Figures 5c and 5f) showed that a few proteins were eluted at the same retention time as the first population of pectins (from 6.6 to 8.4 ml). This population represented 27 and 28% of the total injected polysaccharides for genotypes RG822 and NV9392, respectively. The intrinsic viscosity of this population was 332 and 376 ml/g for genotypes RG822 and NV9392, respectively. The second population of the  $P_H$  was eluted between 8.4 and 9.7 ml. It represented 41 and 42% of the total injected polysaccharides for genotypes RG822 and NV9392, respectively. The intrinsic viscosity of this population was 83 and 104 ml/g for genotypes RG822 and NV9392, respectively. Finally, the third population of the  $P_H$  was eluted between 9.7 and 10.8 ml. It represented 26 and 25% of the total injected polysaccharides for genotypes RG822 and NV9392, respectively. The intrinsic viscosity of this population was 11 and 12 ml/g for genotypes RG822 and NV9392, respectively. The intrinsic viscosity of the whole population of the  $P_H$  was also determined. It was 167 and 199 ml/g for genotypes RG822 and NV9392, respectively. These results were lower than for  $P_W$  and  $P_O$ . The intrinsic viscosities were also low compared to those described in literature for acid-extracted apple pectins (Cros *et al.* 1996, Axelos & Thibault 1991). In contrast to the yields of  $P_W$  and  $P_O$ , the chromatographic yield of  $P_H$  was high (94 and

95% of the total injected polysaccharides for genotypes RG822 and NV9392, respectively).

As the highest quantity of pectins was extracted by dilute acid, the properties of the  $P_H$  were further investigated by anion-exchange chromatography. The profiles obtained are shown in Figure 7. For both genotypes, four main fractions were separated (F1 to F4 and F'1 to F'4 for genotypes RG822 and NV9392, respectively). The recoveries during chromatography were 96 and 95% for the neutral sugars of the  $P_H$  of genotypes RG822 and NV9392, respectively, and 113 and 120% for the GalA constituent of the  $P_H$  of genotypes RG822 and NV9392, respectively. Yields and compositions of the fractions are given in Table 13.

The first fraction represented 29 and 32% of the  $P_H$  of genotypes RG822 and NV9392, respectively. It was mainly composed of neutral sugars (33.4 and 49.3 g/100 g F1 and F'1, respectively). Ara and Gal were the main sugars detected, indicating that F1 and F'1 were composed of arabinans, galactans and/or arabinogalactans.

The second fraction represented 16 and 19% of the  $P_H$  of genotypes RG822 and NV9392, respectively. It was mainly composed of GalA (57.2 and 64.5 g/100 g F2 and F'2 respectively). Its UA/Rha molar ratio (161 and 182 for F2 and F'2, respectively) and its DM (93 and 82 for F2 and F'2, respectively) were high, indicating that this population was mainly composed of highly methylated homogalacturonans.

The third fraction was the main one. It represented 32 and 45% of the  $P_H$  of genotypes RG822 and NV9392, respectively. It was also mainly composed of GalA (71.8 and 75.6 g/100 g F3 and F'3, respectively) but had a higher rate of Rha than the F2 and F'2 fractions. The molar ratio of UA/Rha was 25 and 27 for F3 and F'3, respectively, indicating that these fractions contained a higher proportion of rhamnogalacturonans than F2 and F'2. The DM was lower than for F2 and F'2 (52 and 58 for F3 and F'3, respectively).

The  $P_H$  of the two genotypes was very similar regarding the yields and compositions of the first three fractions. The fourth fraction was, however, different for the two genotypes. It represented 23% of the  $P_H$  of genotype RG822 whereas it represented only 4% of the  $P_H$  of genotype NV9392. F4 was mainly composed of GalA (71 g/100 g F4), whereas GalA represented only 35% of F'4. The DM of F4 and F'4 were 18 and 38, respectively, and the DAc were 2 and 4, respectively.

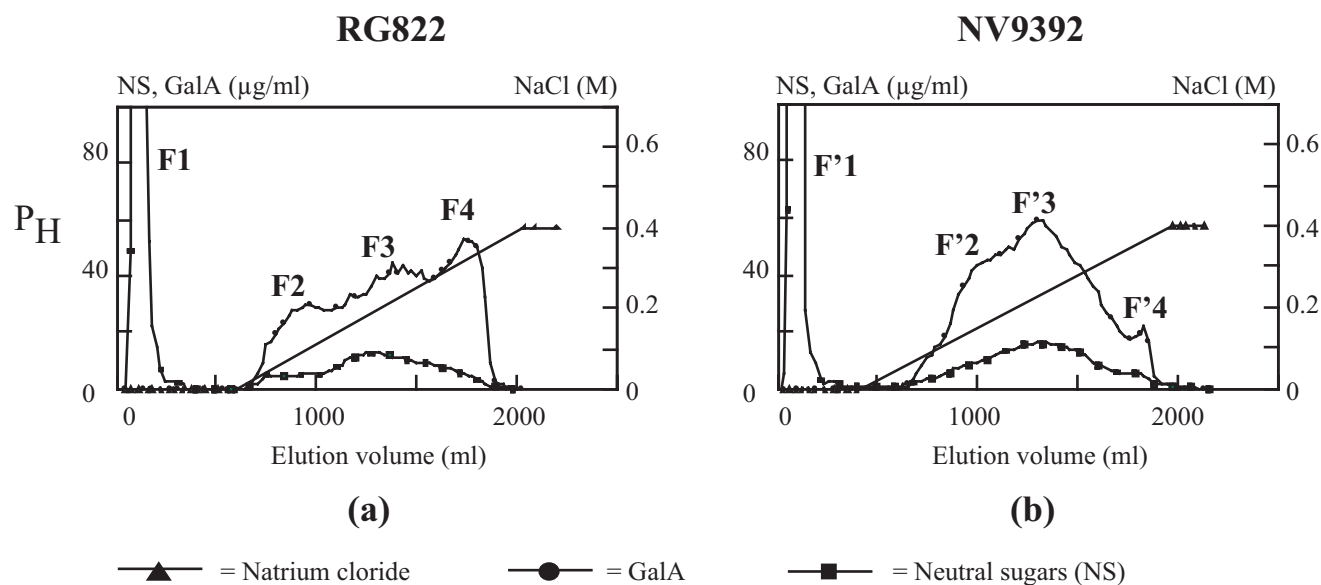
## DISCUSSION

The different parts of the fruits of Japanese quince contained large amounts of dietary fibre. The fibre from the flesh represented an average of 53 g per 100 g total extracted fibre of the dry fruit, whereas that from the skin and the carpel zones represented 9 and 38 g, respectively.

Although the two analytical methods (AIS and AOAC) resulted in significantly different values, no interaction between genotype and method was found. Any method could thus be used to screen chaenomeles fruits for dietary fibre and produce a valid ranking of genotypes. The fibre content varied from 28 to 38 g per 100 g dry matter, depending on genotype. This is a considerable variation, which must be taken into

**Table 12.** Intrinsic viscosity of pectins  $P_w$  and  $P_o$  extracted from fruits of two Japanese quince (*C. japonica*) genotypes, RG822 and NV9392.

Pectins	Genotype	$[\eta]$ (ml/g)	$\lambda_H$	$\lambda_K$
$P_w$	RG822	804	0.71	0.08
$P_w$	NV9392	647	0.75	0.06
$P_o$	RG822	564	0.35	0.14
$P_o$	NV9392	687	0.43	0.12



**Figure 7.** Elution profiles of the pectin  $P_H$ , extracted from entire frozen fruits of Japanese quince (*C. japonica*) genotypes RG822 (a) and NV9392 (b), on a DEAE Sepharose CL-6B column, eluted by a 50 mM sodium succinate buffer (pH 4.5) and a linear gradient of NaCl in 50 mM sodium succinate buffer.

account when selecting varieties for fruit production, manufacture of fibre-rich food products and pectins. As the fruits were sampled at an apparently equivalent stage of development, it is likely that most of the variation revealed was due to genotypic differences. However, to fully investigate the environmental contribution to the variation it would be necessary to sample fruits from plants grown in replicated field trials. Furthermore, it is interesting to note that the sample of flowering quince (*C. speciosa*) contained the largest amount of fibre. More genotypes of this species would need to be studied before it is possible to draw any conclusions about the significance of this finding.

The study of the cell-wall polysaccharides of Japanese quince was performed in two stages: i) preparation of AIS and ii) sequential extraction of AIS. The AIS method is commonly used to isolate cell-wall

**Table 13.** Yield and composition of sugars in fractions obtained by anion exchange chromatography of pectin  $P_H$ , extracted from fruits of Japanese quince (*C. japonica*) genotypes RG822 and NV9392.

Fraction	Yield <sup>a</sup>	Sugar composition <sup>b</sup>										
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc <sub>t</sub>	GalA	Tot.	DM	DAC
RG822												
F1	29	0.3	0.3	27.6	0.8	0.4	3.3	0.7	1.2	34.6	nd	nd
F2	16	0.3	0.1	0.7	0.3	0.3	0.7	0.5	57.2	60.1	93	3
F3	32	2.2	0.2	1.6	1.0	0.2	2.2	0.6	63.8	71.8	52	7
F4	23	1.3	0.1	0.7	0.4	0.2	0.8	0.4	71.0	74.9	18	2
NV9392												
F'1	32	0.4	0.1	38.9	1.0	0.7	6.1	2.1	1.7	51.0	nd	nd
F'2	19	0.3	0.1	0.7	0.4	0.3	0.8	0.5	64.5	67.6	82	4
F'3	45	2.1	0.2	1.6	0.8	0.2	2.1	0.8	67.8	75.6	58	6
F'4	4	1.4	0.0	0.9	0.7	0.6	1.1	0.9	35.0	40.6	38	4

<sup>a</sup>g/100 g  $P_H$ , <sup>b</sup>g/100 g fraction, Glc<sub>t</sub> = total glucose, nd = not determined



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 *et al.* 1985, Selvendran & O'Neill 1987). The proteins in the AIS (4–12%) could be ascribed to cell-wall proteins or co-precipitated intracellular proteins. The extraction scheme 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, 8 g hemicelluloses and 60 g cellulosic residue/100 g AIS. Every 100 g entire dry fruit (800g entire fresh fruit) contained 11 g pectins, 3 g hemicelluloses and 18 g cellulosic residue. The recovery of polysaccharides was high (86.8% and 91.9% for genotypes RG822 and NV9392, respectively), indicating that little material was lost during the extraction. It is also important to note that: 1) pectins were mostly located in the flesh of the fruits, 2) the core mostly contained cellulose and 3) the skin was quite rich in non-fibre compounds (such as lipids and proteins). In terms of content of polysaccharides and their composition, there was no apparent difference between the two genotypes studied.

Sequential extraction is generally used for fractionation of pectins. Following water extraction,  $\text{Ca}^{++}$  chelators such as ethylenediamine tetraacetate (EDTA) or cyclohexane-trans-1,2 diamine tetraacetate (CDTA), are often used to extract pectins. However, it has been shown (Mort *et al.* 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, 25 °C) solubilised a low amount of pectins (6 and 2 g/100 g AIS for water and oxalate, respectively) but the conditions were not degradative and pectin structure was preserved. Indeed, a very high and unusual intrinsic viscosity was measured for these pectins. Further investigation of their gelling properties and fine structure would be interesting, although the yields were low in these conditions.

By contrast, dilute acid solubilised a high quantity of pectins (17 g/100 g AIS). These extraction conditions were, however, degradative. They are known to break some covalent linkages, especially those involving Ara residues. Indeed, the quantity of Ara residues separated by anion exchange chromatography (F1 and F'1) was significant. The  $P_H$  had a rather low intrinsic viscosity and a lower molar ratio of UA/Rha than the  $P_W$  and the  $P_O$ . This result was in agreement with those described for apple by Massiot *et al.* (1994). Once purified, the main fraction of the  $P_H$  (F3 and F'3) had a molar ratio for UA/Rha, in agreement with that of the  $P_H$ , indicating that they contained a higher proportion of rhamnogalacturonans than the  $P_W$  or the  $P_O$ . The  $P_H$  was composed of four populations, the first (F1 and F'1) being mainly composed of arabinan, the second of homogalacturonans, the third of rhamnogalacturonans and the fourth differing depending on the genotype studied.

Subsequent treatment of the remaining cell-wall with alkali liberated some additional pectins (6 g/100 g AIS), probably through limited  $\beta$ -elimination reactions of methyl galacturonate residues. Simultaneously, most of the methyl ester and acetyl groups were removed. At this stage, not all the pectins were removed from the AIS, its UA content being 5 and 7 g/100 g AIS for genotypes NV9392 and RG822, respectively. These results are similar to those obtained for sugar beet pulp (Rombouts & Thibault 1986 and Bertin *et al.* 1988).

Pectins extracted under non-alkaline conditions ( $P_W$ ,  $P_O$  and  $P_H$ ) had a high degree of methylation and a low degree of acetylation. Pectins extracted by a chelating agent are believed to be complexed mainly with  $\text{Ca}^{++}$  (Selvendran *et al.* 1985). However, oxalate-soluble pectins often have a high content of methyl ester groups (Renard *et al.* 1990, Ros *et al.* 1998) and Table 9 shows that the DM of  $P_O$  was high (> 60%). A complexation through cations would only be possible if the methoxyl groups were block-esterified, which has to be confirmed.

During the extractions, recovery of each sugar was close to 100%. However, Ara and Rha had a substantially lower yield (on average 74 and 73% for Ara and Rha, respectively). Analysis of the sugar composition of polysaccharides involved acidic hydrolysis. The various stabilities of the monosaccharides liberated, as well as those of the different linkages involved, would have required different optimum

conditions for hydrolysis. A long and harsh hydrolysis step destroys some of the Ara, whereas a short and mild hydrolysis underestimates 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 to all samples, as a compromise. Therefore, Rha and Glc may be underestimated in all the extracts. Ara was also underestimated due to the extraction method, which included an acidic step. Indeed, below pH 3, hydrolysis occurred for 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 found in the quantity of polysaccharides extracted from the AIS of the two genotypes or in the composition of these constituent 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 must be analysed to confirm our results.

## CONCLUSION

Fruits of Japanese quince contain high amounts of dietary fibre and pectins. On average 32 g dietary fibre can be extracted from 100 g dry fruit. This is higher than from apple, for which only about 13 g can be extracted from 100 g dry fruit (Massiot *et al.* 1994). On average 11 g pectins can be extracted from 100 g dry fruit (corresponding to 1.4 g/100 g fresh fruit) of Japanese quince. This is higher than or similar to apples, from which 0.5 to 1.6 g pectins can be extracted per 100 g fresh fruit (Thakur *et al.* 1997). The pectins extracted from fruits of Japanese quince may have valuable properties as indicated by their high intrinsic viscosity, high DM and low DAC. Further investigations should be devoted to determining the fine structure of the pectins and characterising their physico-chemical properties in detail, including their gelling properties.

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