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# Pectins in the fruits of Japanese quince (*Chaenomeles japonica*)

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**Abstract**

The pectin content, composition and physico-chemical properties were studied in the fruits of two genotypes of Japanese quince. On average, the fruits contained 11 g pectins/100 g dry fruit and 1.4 g pectins/100 g fresh fruit. A sequential extraction was used to isolate the pectins from the fruits. The cells from the flesh were examined using a confocal laser scan microscope, fresh and after each extraction step of the sequence. The dilute acid conditions were the most efficient for pectin extraction. Pectins extracted by water or potassium oxalate had higher (>600 ml/g) intrinsic viscosities than the pectins extracted by dilute acid (<400 ml/g). Anionic exchange chromatography was performed on the acid-extracted pectins. They were constituted of four populations, the first one being mainly composed of arabinans, the second one of homogalacturonans, the third one of rhamnogalacturonans. The composition of the last one varied with the genotype studied.


*Keywords:* *Chaenomeles*; Japanese quince; Pectins; Pectin extraction; Confocal laser scan microscope; Pectin composition; Pectin intrinsic viscosity; Anion exchange chromatography of pectins

## 1. Introduction

Japanese quince (*Chaenomeles japonica*) is a fruit crop interesting for its acidic juice and aroma (Lesinska, Przybylski, & Eskin, 1988). Our previous work was devoted to the cell-wall polysaccharides of the different tissue-zones and entire freeze-dried fruits of *C. japonica*. It was shown (Thomas, Crépeau, Rumpunen, & Thibault, 2000; Thomas & Thibault, 2002) that Japanese quince contained large amounts of dietary fibres (32 g/100 g dry fruit) and pectins (11 g/100 g dry fruit). The pectin content of the fruits was as high as that of apple (Thakur, Singh, & Handa, 1997; Thomas & Thibault, 2002). Due to their gelling properties, pectins are often used as an additive in the food industry. It is therefore of interest to determine some of the chemical and physico-chemical properties of the Japanese quince pectins. In this study, pectins were extracted from fresh fruits. Two genotypes were chosen according to our previous work (Thomas et al., 2000), one (NV9392) with a high amount of fibre (35 g/100 g dry fruit) and the other (RG822) containing a medium amount of fibre (29 g/100 g dry fruit). The different fresh tissues were observed by confocal laser scan microscopy (CLSM) and the cells from the flesh were examined after each extraction step of the sequential extraction. The chemical composition and some of the physico-chemical properties of the extracted pectins were 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 Agricultural Sciences, Kristianstad, Sweden). The collection was gathered from partly domesticated populations in commercial orchards, or from botanical gardens. Genotypes NV9392 and RG822 were chosen for a detailed study of their cell-wall polysaccharides. All fruits were picked at the same developmental stage, when the seeds in the fruits had turned brown, indicating fruit maturity. Entire fruits were frozen immediately after picking.

The different tissue-zones of the fruits (flesh, carpels and skin) were manually separated and stored frozen until they were studied by microscopy (Section 3.1).

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### 2.2. Preparation of alcohol-insoluble solids

Frozen fruit materials (10 g of entire fruit) were cut into small pieces (~5 mm diameter) in a mortar. The seeds were removed and the fruits pieces (still frozen) were homogenized in 400 ml boiling ethanol (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 of pectins

Sequential extraction of pectins from alcohol-insoluble solids (AIS) 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 three times. AIS was first treated with water at 25 °C for 30 min (pH was adjusted to 4.5 with 0.1 mol/l 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 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 three times with 1% potassium oxalate (adjusted to pH 4.5 with 1 mol/l 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 mol/l, 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 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 washed with 50% ethanol until the conductivity of the filtrate was less than 10 μS. It was dried by solvent exchange, stored one night at 40 °C and 12 h at 40 °C under vacuum.

### 2.4. Chemical analysis

All values are on a dry weight basis and analysis were performed at least in duplicates.

#### 2.4.1. Moisture

The moisture of the fruits and 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.

#### 2.4.2. Neutral sugar

All samples were hydrolysed in 1 mol/l $H_2SO_4$ (3 h, 100 °C) for measurement of individual neutral sugars (Englyst & Cummings, 1984), with an additional pre-treatment with 13 mol/l $H_2SO_4$ (1 h, 25 °C) for insoluble materials (AIS and residues) (Seaman, Moore, Mitchell, & Millet, 1954). The sugars were reduced to their corresponding alditols by adding 3 mol/l $NH_3$ 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 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 GPC 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 (AIS and residues) were submitted to a 1 h prehydrolysis with 13 mol/l $H_2SO_4$ at 25 °C followed by a 3 h hydrolysis with 1 mol/l $H_2SO_4$ at 100 °C. Soluble samples (0.1 ml of 1 mg/ml pectin solutions) were treated with 0.05 mol/l NaOH (1.9 ml) for 30 min at room temperature and neutralized with 0.1 mol/l HCl (1 ml) 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 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 (2002). Five milligrams of pectin were saponified during 2 h at room temperature in 1 ml of a 0.4 mol/l NaOH solution in 80% isopropanol. 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 cm × 0.4 cm) column thermostated at 25 °C and equipped with a Merck C18 guard cartridge (0.4 cm × 0.4 cm). The eluant was $4 \times 10^{-3}$ mol/l $H_2SO_4$ (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 GalA.

### 2.5. Physico-chemical analysis

#### 2.5.1. Anion exchange chromatography

Solutions (50 ml) of pectins (2 mg/ml) were loaded on a column (37 cm × 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 mol/l sodium succinate buffer (pH 4.5) at 1.27 ml/min. Pectic material bound to the gel was then eluted by increasing the ionic strength of the buffer. A linear gradient (1500 ml) from 0 to 0.4 mol/l NaCl in 0.05 mol/l sodium succinate buffer pH 4.5 was applied to the column. The column was then washed with 380 ml of 0.4 mol/l NaCl in 0.05 mol/l sodium succinate buffer pH 4.5. The fractions (12.7 ml) were assayed for GalA and neutral sugars and pooled. The purified fractions were extensively dialysed against deionised water and freeze-dried.

#### 2.5.2. High-performance size-exclusion chromatography and viscosity measurements

Pectins (5 mg/ml) were solubilised one night at room temperature under gentle shaking in a 0.05 mol/l $NaNO_3$ solution containing 0.02% $NaN_3$. They were then filtered through a 0.45 μm membrane (Millipore, Millex, HV). Fifty microlitres of the filtered solution were loaded on the high-performance size exclusion chromatography (HPSEC)-Viscotek system. HPSEC was performed at 25 °C on a PL aquagel-OH mixed 8 μm column (Polymer laboratories, 300 cm × 7.5 mm) equipped with a PL aquagel-OH 8 μm guard column (Polymer laboratories, 50 cm × 7.5 mm) eluted at 1 ml/min with 0.05 mol/l $NaNO_3$ containing 0.02% $NaN_3$. The column was mounted in series with an UV detector (SpectraSERIES UV100) and with a parallel-coupled RI (ERC 7517A) and Viscotek (T-50A, Viscotek) detectors. Data acquisition was done, using the Trisec (Viscotek) software.

Viscometry measurements were also made using an Ubbelhode capillary viscometer (0.46 mm) thermostated at 25 °C. Pectins (2 mg/ml) were solubilised one night at room temperature under gentle shaking in a 0.05 mol/l $NaNO_3$ solution containing 0.02% $NaN_3$. They were then filtered through a 0.45 μm membrane (Millipore, Millex, HV). Dilutions ($C$/2, $C$/3, $C$/4 and $C$/6) were achieved in 0.05 mol/l $NaNO_3$ containing 0.02% $NaN_3$. Intrinsic viscosity was calculated by extrapolating to $C = 0$ the Huggins (1) and Kraemer (2) equations:

$$\eta_{red} = \frac{\eta_{spe}}{C} = [\eta] + \lambda_H C[\eta]^2 \quad (1)$$

$$\eta_{inh} = \ln\left(\frac{\eta_r}{C}\right) = [\eta] - \lambda_K C[\eta]^2 \quad (2)$$

with:

$\eta_r$ relative viscosity,
$\eta_{inh}$ inherent viscosity,
$\eta_{red}$ reduced viscosity,
$\eta_{spe}$ specific viscosity,
$C$ concentration (g/ml),
$[\eta]$ intrinsic viscosity (ml/g)
$\lambda_H$ Huggins coefficient,
$\lambda_K$ Kraemer coefficient.

#### 2.5.3. Microscopy

Handcut thin sections (about 1 mm) of fruit-tissues (flesh, carpels and skin) were coloured (10 min) with 0.02% acridine orange (CI 46005) in a 0.1 mol/l phosphate buffer (pH 7). Confocal 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 × /1.2 water-immersion objective. A 488 nm argon ion laser was used to excite the dye. A Long Pass filter (LP 515) allowed to collect fluorescence emission higher than 515 nm. Attenuation was set to 30 for the study of the cell-wall during the sequential extraction of the pectins. It was increased to 100 for the observation of the unstained flesh tissue and to 1000 for the observation of the wax layer covering the skin. The same conditions of contrast and brightness were used for all the observations. Sequences of 25 $x-y$ optical sections were collected at increments of 1 μm in the $z$-axis. Images were captured by the Carl Zeiss LSM software.

## 3. Results and discussion

### 3.1. Microscopic observation of the initial samples

The different tissue-zones of genotype RG822 were first observed in their initial state (Fig. 1(a)–(f)). Fig. 1(a) and (b) shows 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 (Fig. 1(b)). 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 (indicated by an arrow), their number probably depending on the fruit maturity.

Fig. 1(c) and (d) shows 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 carpels have an elongated shape. Their length varies from 90 to 180 μm and their width is about 18 μm. The sample was taken in

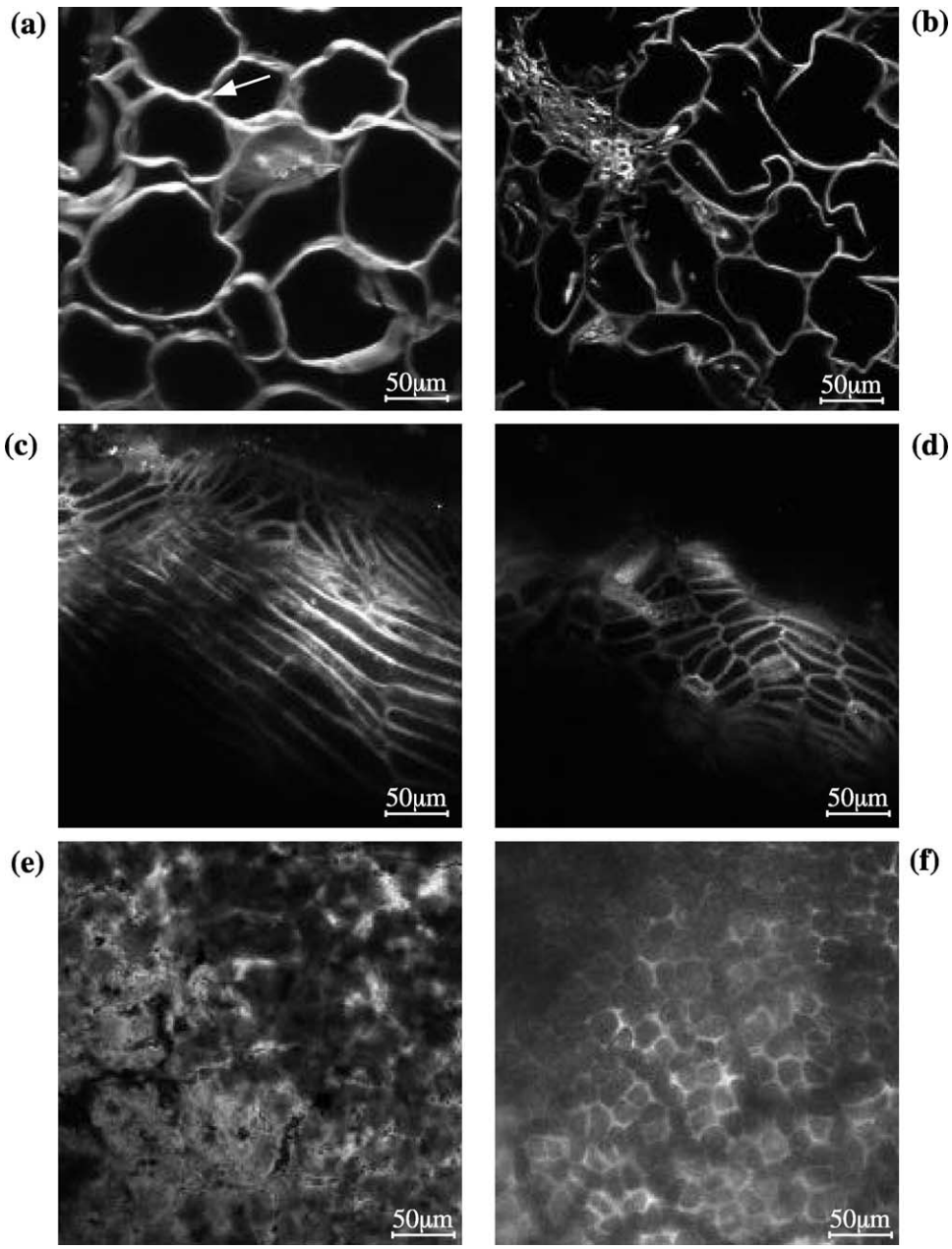


Fig. 1. Images of the different tissue-zones of the fruits observed with a CLSM (40 × /1.2). Samples were stained with acridine orange.
(a): flesh, 25 focal planes, C 369, B9845, At 30, P 8.
(b): flesh, 1 focal plane, C 369, B9845, At 100, P 8.
(c): carpels, C 369, B9845, At 10, P 8.
(d): carpels, same focal plane as (c) (variation on the x-y axis), C 369, B9845, At 30, P 8.
(e): skin observed on the external surface, 1 focal plane, C 369, B9845, At 1000, P 8.
(f): 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

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. Fig. 1(e) and (f) shows two views of the same sample of fruit skin. The outer surface is covered by a wax layer that has no specific organization (Fig. 1(e)). The cells of the skin can be seen on the inner surface of the skin (Fig. 1(f)); they have a polyhedral shape of about 25 µm diameter and have a thick cell-wall. No intercellular spaces can be observed.

### *3.2. Microscopic observations during the extraction of pectins*

The flesh, the major part of the fruit (Thomas et al., 2000), was chosen for the observation of the cell-wall

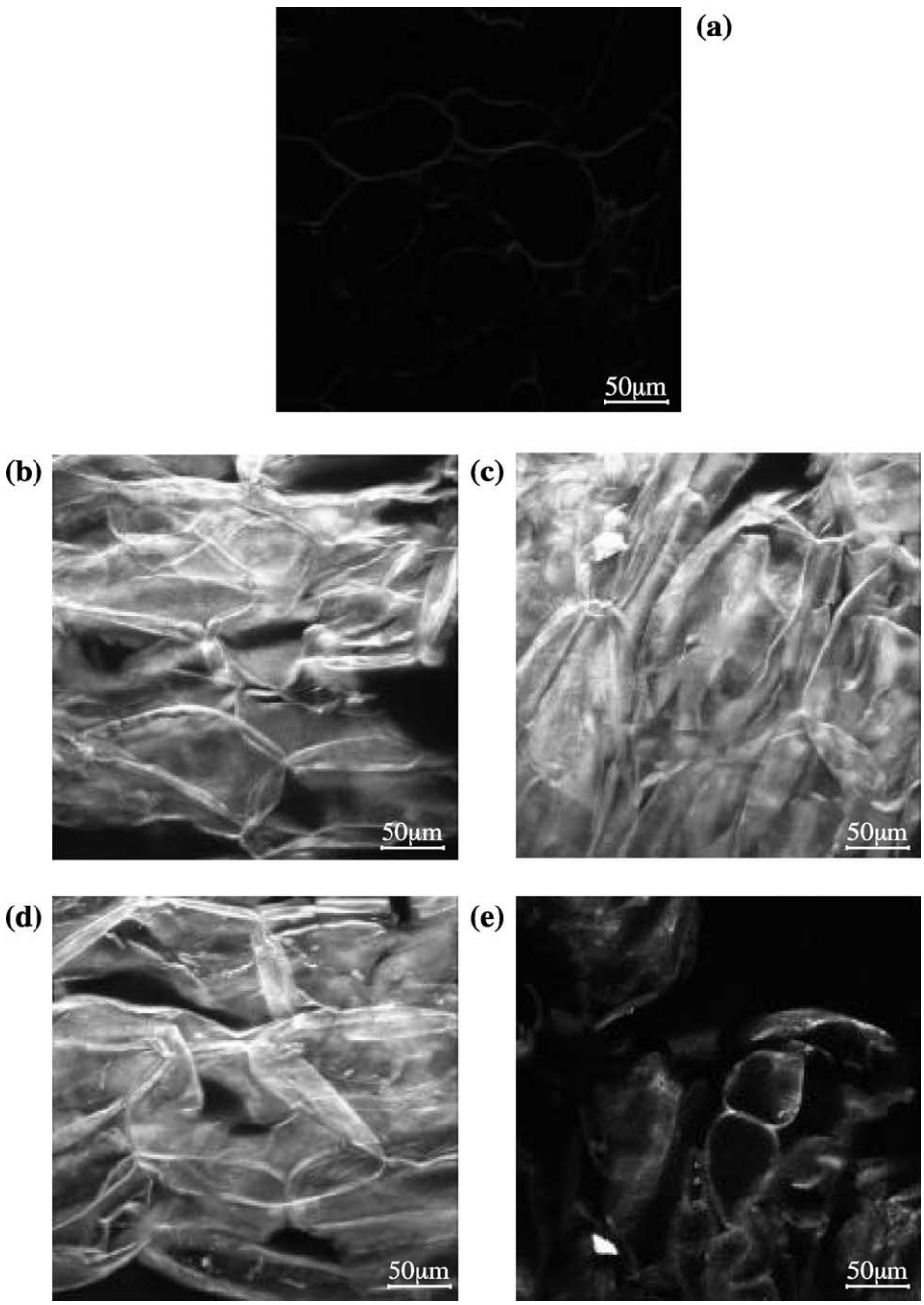


Fig. 2. Images of the flesh of the fruits observed with a CLSM (40 × /1.2) during the sequential extraction of pectins. Samples (except (a)) were stained with acridine orange.
(a): flesh unstained, 1 focal plane, C 369, B 9848, At 100, P 8.
(b): flesh after preparation of the AIS, 25 focal planes, C 369, B 9845, At 30, P 8.
(c): flesh cells after water extraction of the pectins, 25 focal planes, C 369, B 9845, At 30, P 8.
(d): flesh cells after oxalate extraction of the pectins, 25 focal planes, C 359, B 9845, At 30, P 8.
(e): 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

during pectin extraction (Fig. 2(a)–(e)). An unstained sample of the flesh tissue was observed (Fig. 2(a)). There was no autofluorescence indicating that no or very few phenolic compounds were present in the cell-wall of the flesh. Fluorescence in the following figures was thus a result of the staining with acridine orange. Acridine orange is a metachromatic dye employed as a fluorochrome to display the presence of anions (Conn's, 1977). It was chosen as an indicator for the presence of pectins in the cell-wall.

The cells in the AIS had a distorted shape (Fig. 2(b)) probably due to the 20 min boiling in ethanol. The quantity

of intercellular spaces was not increased and fluorescence with acridine orange was still important.

After water (Fig. 2(b)) and oxalate (Fig. 2(c)) extraction, the topology of the tissue was very similar to that of the AIS (Fig. 2(a)). The cells showed a distorted shape, the number of intercellular spaces was not increased and fluorescence with acridine orange was still important indicating that pectins were still present in the residue.

The topology of the tissue after acidic extraction (Fig. 2(d)) was completely different from that obtained after preparation of the AIS (Fig. 2(a)), water extraction (Fig. 2(b)) or oxalate extraction (Fig. 2(c)); the cells were seldom bound to each other and the intensity of the fluorescence of the material stained with acridine orange has dramatically decreased.

### 3.3. Yield and composition of the AIS

AIS content and composition of the entire fruits of two frozen genotypes of *C. japonica* can be seen in Table 1. In the whole fruit, the AIS represented 35.5 and 38.4 g/100 g dry fruit for the frozen genotypes RG822 and NV9392, respectively. These results are similar to those obtained for apple by Massiot, Baron, and Drilleau (1994) and to those we obtained previously on freeze-dried fruits of *Chaenomeles* (Thomas & Thibault, 2002).

Total (acidic and neutral) sugars contents of the AIS determined from the entire fruits were ~72 g total sugars/100 g AIS of RG822 and ~76 g total sugars/100 g AIS of NV9392 (Table 1). It corresponded to 26 and 29 g total sugars/100 g dry matter in the whole dry fruits for genotypes RG822 and NV9392, respectively. Other components were the substituents of the pectins (acetic acid, methanol), proteins and some non-sugar cell-walls constituents such as polyphenols or lignin may also be present.

The carbohydrate polymers of the AIS were mostly pectic polysaccharides and glucans. Neutral sugars represented 51 and 54 g/100 g AIS in the entire fruits of genotypes RG822 and NV9392, respectively. The main neutral sugars in the AIS were Glc (27–28 g/100 g AIS), Xyl (7–6 g/100 g AIS), Ara (9–10 g/100 g AIS) and Gal (5–6 g/100 g AIS). Acidic sugars represented 21 and 22 g/100 g AIS. Among them, GlcA represented less than 5% of total acidic sugars (results not shown). Acidic sugars were thus assimilated to GalA. 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) and were also in agreement with our previous study on *Chaenomeles* (Thomas & Thibault, 2002).

### 3.4. Extraction of pectins: their yields and compositions

After 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 final residue was dried and weighed. Yields and compositions of the pectins extracted with water, potassium oxalate or dilute acid can be seen in Table 2.

#### 3.4.1. Yields

Pectins extracted from the AIS with water ($P_W$) represented 4.4 and 3.7 g/10 g AIS for genotypes RG822 and NV9392, respectively. A small and additional quantity of pectins ($P_O = 1.7$ and 1.9 g/100 g AIS for the two same genotypes) was extracted by potassium oxalate from the residues of the water extraction. A further extraction step, performed with hot dilute acid on the residues of the oxalate extraction, yielded an important quantity of pectins ($P_H = 26.5$ and 23.7 g/100 g AIS for the two same genotypes). The final residue represented 56.1 and 59.2 g/100 g AIS for genotypes RG822 and NV9392, respectively. The sum of the weight of the extracts and the final residues was higher than 85%, indicating that only few material was lost during extractions. The pectins ($P_W + P_O + P_H$) represented 32.6 and 29.3 g/100 g AIS for genotypes RG822 and NV9392, respectively, hot dilute acid being the most efficient extractant for pectins (81% of total extracted pectins were solubilised at this step). No significant difference in pectin content was observed between the two genotypes (11.6 and 11.3 g pectins/100 g dry fruit of genotypes RG822 and NV9392, respectively), whereas genotype NV9392 contained a higher proportion of cellulosic residue (22.7 g/100 g dry fruit) than genotype RG822 (19.9 g/100 g dry fruit). These results are very close to those obtained in our previous work on *Chaenomeles* (Thomas & Thibault, 2002).

Table 1
AIS content and composition of two genotypes of Japanese quince

| Genotype | Dry matter[a] | Yield[b] | Sugar composition[c] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Rha | Fuc | Ara | Xyl | Man | Gal | Glc | GalA |
| RG822 | 10.6 | 35.5 | 1.2 | 0.6 | 8.8 | 6.5 | 1.7 | 5.1 | 27.2 | 21.1 |
| NV9392 | 11.7 | 38.4 | 1.1 | 0.6 | 9.9 | 6.2 | 2.2 | 5.7 | 27.8 | 22.3 |

[a] g/100 g fresh fruit.
[b] g/100 g dry fruit.
[c] g/100 g AIS.

Table 2
Yields and compositions of the pectins and residues obtained from two genotypes of Japanese quince

| Genotype | Extract | Yield[a] | Sugar composition[b] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Rha | Fuc | Ara | Xyl | Man | Gal | Glc | GalA | DM | DAc |
| RG822 | $P_W$ | 4.4 | 1.6 | 0.3 | 7.1 | 1.3 | 0.7 | 6.1 | 2.0 | 61.3 | 72 | 6 |
| | $P_O$ | 1.7 | 1.1 | 0.2 | 5.8 | 0.5 | 0.4 | 5.9 | 0.9 | 57.5 | 57 | 4 |
| | $P_H$ | 26.5 | 1.7 | 0.2 | 15.2 | 1.1 | 0.1 | 4.8 | 0.8 | 53.0 | 55 | 5 |
| | Residue | 56.1 | 0.8 | 0.4 | 1.0 | 11.8 | 2.4 | 4.2 | 43.4 | 7.0 | nd | nd |
| NV9392 | $P_W$ | 3.7 | 1.2 | 0.4 | 8.2 | 3.0 | 1.1 | 9.0 | 5.1 | 53.6 | 74 | 6 |
| | $P_O$ | 1.9 | 0.8 | 0.2 | 4.6 | 0.9 | 0.5 | 4.4 | 1.5 | 62.8 | 59 | 4 |
| | $P_H$ | 23.7 | 1.4 | 0.3 | 20.1 | 1.4 | 0.3 | 5.8 | 1.5 | 56.0 | 58 | 5 |
| | Residue | 59.2 | 0.7 | 0.3 | 0.9 | 14.4 | 2.4 | 3.7 | 39.7 | 5.7 | nd | nd |

nd, not determined.
[a] g/100 g AIS.
[b] g/100 g extract.

### 3.4.2. Compositions

The $P_W$ fractions contained 61 and 64 g GalA/100 g extract for genotypes RG822 and NV9392, respectively. They were highly esterified (DM ~ 73) and their Dac was low (~6). Next to GalA residues, the $P_W$ consisted of 19 and 28 g neutral sugars/100 g $P_W$; the major ones being Ara (7.1 and 8.2 g/100 g extract for the same genotypes) and Gal (6.1 and 9.0 g/100 g extract for the same genotypes). Small amounts of Glc were also present in the $P_W$ (2 and 5 g/100 g AIS for genotypes RG822 and NV9392, respectively). Total neutral and acidic sugars in these pectins represented an average of 81 g/100 g extract. Other constituents may be substituents of the pectins (methanol, acetic acid) and proteins. Water soluble pectins extracted from Japanese quince contained higher amounts of Ara and Gal than those extracted from sugar beet (Fares, Renard, R'zina, & Thibault, 2001). They had a GalA/Rha molar ratio equivalent to 32 and 38 for genotypes RG822 and NV9392, respectively, which was higher than that obtained for sugar beet and lemon as described by Fares et al. (2001) and Ralet and Thibault (1994), respectively. This indicated that in Japanese quince, the water-soluble pectins contained lower proportions of rhamnogalacturonic regions than sugar beet or lemon.

Pectins extracted by oxalate from Japanese quince contained galactose, arabinose, rhamnose, xylose and a little fucose, as reported for water soluble pectins. Total neutral and acidic sugars in the pectins represented an average of 74 g/100 g extract. Other constituents may be substituents of the pectins (methanol, acetic acid) and proteins. The degree of methylation of the $P_O$ (58, on average) was lower than that of the $P_W$ (73, on average) and the acetylation degree was low (4). Acidic sugars represented 58 and 63 g/100 g extract and neutral sugars 15 and 13 g/100 g extract for genotypes RG822 and NV9392, respectively; the major ones being Ara (5.8 and 4.6 g/100 g extract for the same genotypes) and Gal (5.9 and 4.4 g/100 g extract for the same genotypes). The $P_O$ contained lower amounts of Ara and Gal than the $P_W$ and their GalA/Rha molar ratios (44 and 66 for genotypes RG822 and NV9392, respectively) were also higher, indicating that the $P_O$ contained lower proportions of side-chains carrying rhamnogalacturonic regions than the $P_W$ and also than sugar beet (Fares et al., 2001) or lemon (Ralet & Thibault, 1994).

Table 2 showed that total neutral and acidic sugars in the $P_H$ amounted an average of 82 g/100 g extract. Other constituents may be substituents of the pectins (methanol, acetic acid) and proteins. The DM of the $P_H$ (57, on average) was similar to that of the $P_O$ and lower than that of the $P_W$ and the DAc was low (5, on average). Acidic sugars represented 53 and 56 g/100 g extract and neutral sugars 24 and 31 g/100 g extract for genotypes RG822 and NV9392, respectively. Ara was the main neutral sugar in the $P_H$ (15.2 and 20.1 g/100 g extract for genotypes RG822 and NV9392, respectively). The GalA/Rha molar ratios (26 and 33 for genotypes RG822 and NV9392, respectively) were lower than that of the $P_W$ or $P_O$, indicating that the $P_H$ contained higher proportions of side-chains carrying rhamnogalacturonic regions than the $P_W$ or $P_O$.

## 3.5. Residue

The compositions of the final residues were determined (Table 2). They were mainly composed of neutral sugars (64 and 62 g/100 g residue for genotypes RG822 and NV9392, respectively), Glc being the main one (43 and 40 g/100 g residue for genotypes RG822 and NV9392, respectively). The two residues also contained high levels of Xyl (12 and 14 g/100 g residue for genotypes RG822 and NV9392, respectively), probably coming from xylans. It can be noticed that the residues still contained some Rha and GalA (0.8 and 0.7 g Rha/100 g residue and 7.0 and 5.7 g GalA/100 g residue for genotypes RG822 and NV9392, respectively), indicating that some pectins still remained in the residues after the sequential extraction. This result was in

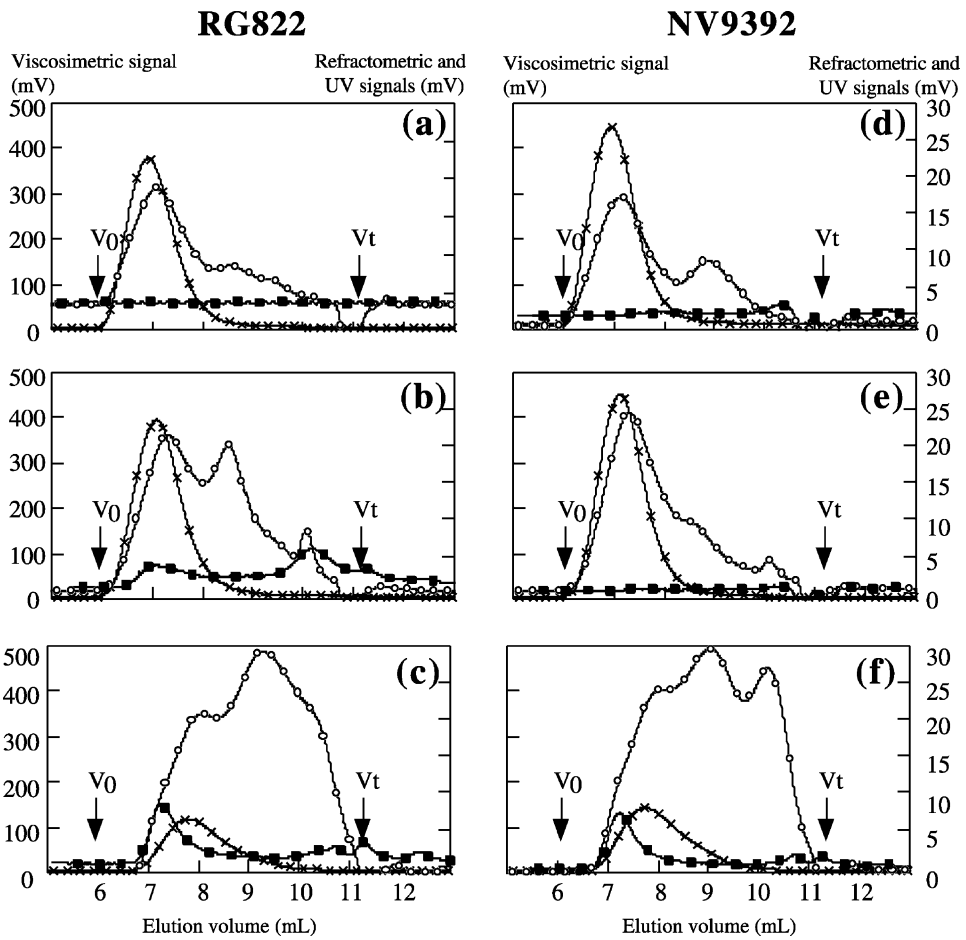


Fig. 3. Viscosimetric, refractometric and UV profiles of pectins: $P_W$ (a and d), $P_O$ (b and e) and $P_H$ (c and f) of entire frozen fruits of genotypes RG822 (a–c) and NV9392 (d–f).
Refractometric signal (mV): —○—, Viscosimetric signal (mV): —×—, UV signal (mV): —■—

agreement with our previous work on *Chaenomeles* (Thomas & Thibault, 2002).

### 3.6. Molar masses of pectins

Molar masses of the $P_W$, $P_O$ and $P_H$ were studied using the HPSEC-Viscotek system. The recovery of the polysaccharides after filtration was higher than 95% (results not shown), indicating that very few polysaccharides was lost during filtration. Refractometric, viscosimetric and UV profiles of the $P_W$, $P_O$ and $P_H$ can be seen on Fig. 3(a)–(c) for genotype RG822 and on Fig. 3(d)–(f) for genotype NV9392. There were no UV-absorbing substances in the $P_W$ fractions and very few in the $P_O$ and $P_H$ fractions.

The chromatographic profiles showed that $P_W$ contained at least two populations. The first one 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 (Table 3). The second population constitutive of the $P_W$ 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 (Table 3). The intrinsic viscosity of the whole population of the $P_W$ has also been calculated. It was 1109 and 1071 ml/g for genotypes RG822 and NV9392, respectively. These results were very high compared to those described by Chou and Kokini (1987) for tomato pastes water-extracted pectins (340 ml/g). Moreover, the chromatographic yields were low (32 and 47% of the total injected polysaccharides for genotypes RG822 and NV9392, respectively). Some polysaccharides may thus

Table 3
Intrinsic viscosity of the $P_W$ and $P_O$ of genotypes RG822 and NV9392 measured with an Ubbelhode capillary viscometer

| 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 |

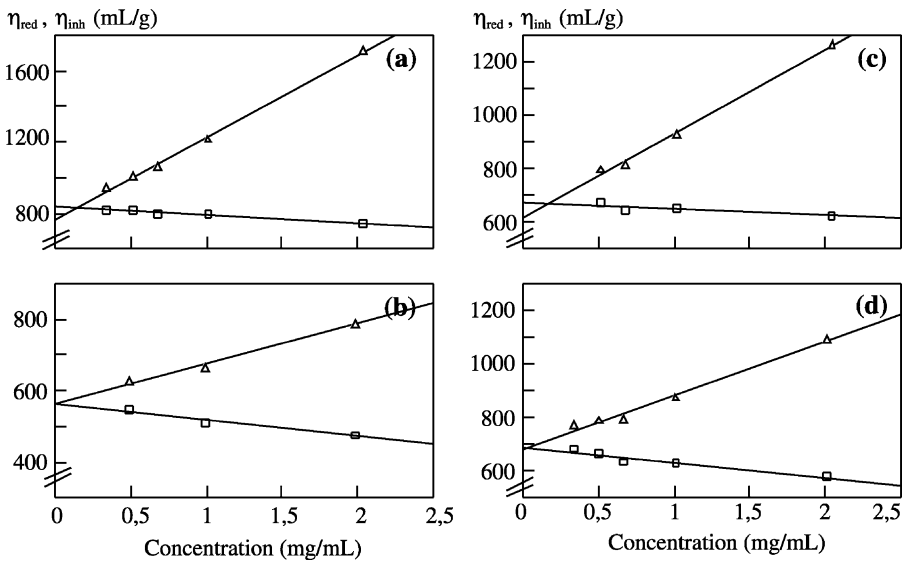


Fig. 4. Intrinsic viscosity of the $P_W$ (a and c) and $P_O$ (b and d) of genotypes RG822 (a and b) and NV9392 (c and d), reduced viscosity (mL/g): —△—, inherent viscosity (mL/g): —□—.

have remained bound to the column. Therefore, intrinsic viscosity of the $P_W$ was also measured with an Ubbelohde capillary viscometer (Fig. 4(a) and (c)). Results (Table 3) confirmed that the intrinsic viscosity was very high (804 and 647 ml/g for genotypes RG822 and NV9392, respectively). Moreover, the Huggins and Kraemer coefficients were close to 0.5 and 0.05, respectively, indicating that $NaNO_3$ was a good solvent for these pectins.

The $P_O$ were constituted of three populations (Fig. 3). The first one 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 (Table 3). The second population constitutive of the $P_O$ 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 (Table 3). The intrinsic viscosity of the whole population of the $P_O$ was 641 and 840 ml/g for genotypes RG822 and NV9392, respectively. These results were lower than that obtained for the $P_W$. However, they were very high compared to those described in the literature for apple pectins extracted in mild conditions: from 680 to 760 ml/g (Renard & Thibault, 1993). As well as for the $P_W$, the chromatographic yields were low (58 and 42% of the total injected polysaccharides for genotypes RG822 and NV9392, respectively). Therefore, intrinsic viscosity of the $P_O$ was also measured with an Ubbelohde capillary viscosimeter (Fig. 4(b) and (d)). Results (Table 3) showed that the intrinsic viscosity was very high (564 and 687 ml/g for genotypes RG822 and NV9392, respectively) and higher than those described in the literature for apple pectins. Again, the Huggins and Kraemer coefficients were close to 0.5 and 0.05, respectively, indicating that $NaNO_3$ was a good solvent for these pectins.

The $P_H$ were constituted of three populations of pectins, the first one (from 6.6 to 8.4 ml) representing 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 (Table 3). The second population constitutive 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 (Table 3). Finally, the third population constitutive 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 (Table 3). The intrinsic viscosity of the whole population of the $P_H$ has also been determined. It was 167 and 199 ml/g for genotypes RG822 and NV9392, respectively. These results were lower than that obtained for the $P_W$ and $P_O$. Intrinsic viscosity of the $P_H$ was also low compared to values found in the literature for acid-extracted apple pectins (Axelos & Thibault, 1991; Cros, Garnier, Axelos, Imberty, & Perez, 1996). In contrast to the $P_W$ and $P_O$, the chromatographic yields were high (94 and 95% of the total injected polysaccharides for genotypes RG822 and NV9392, respectively).

### 3.7. Anion-exchange chromatography

As the highest quantity of pectins were extracted by the dilute acid, the $P_H$ were further investigated. They

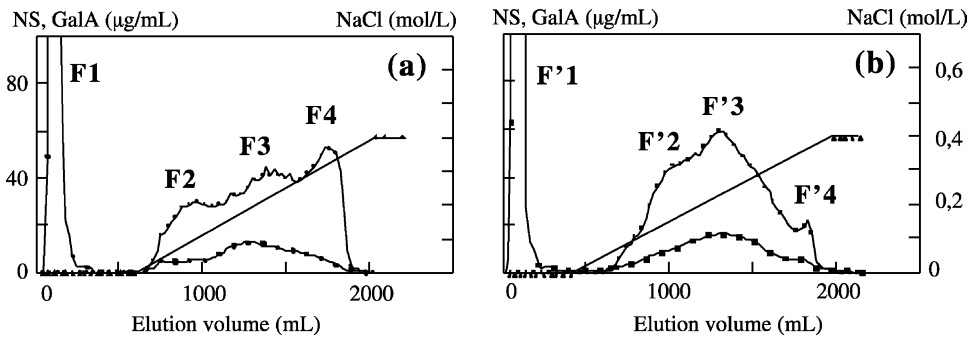


Fig. 5. Elution profiles of the $P_H$ extracted from entire frozen fruits of genotypes RG822 (a) and NV9392 (b) on a DEAE Sepharose CL-6B column, eluted by a 50 mM sodium succinate buffer (pH 4.5) and a linear gradient of NaCl in 50 mM sodium succinate buffer.
NaCl: ▲, GalA: ●, Neutral Sugars: ■

were analysed by an anion-exchange chromatography and their profiles can be seen on Fig. 5. For both genotypes, the profiles were similar: four main fractions were separated (F1–F4 and F′1–F′4 for genotypes RG822 and NV9392, respectively). The recovery yields of the 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 constitutive of the $P_H$ of genotypes RG822 and NV9392, respectively. Yields and compositions of the fractions can be seen in Table 4. A first fraction (F1, F′1) was not bound to the column; it represented 29 and 32% of the $P_H$ of genotypes RG822 and NV9392, respectively, and was mainly constituted 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 may be 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 constituted of GalA (57.2 and 64.5 g/100 g F2 and F′2, respectively). The GalA/Rha molar ratios were very high (161 and 182 for F2 and F′2, respectively), indicating that these fractions contained a very low proportion of rhamnogalacturonic regions. The DM of the F2 and F′2 were very high (93 and 82, respectively) and the DAc were low (3 and 4, respectively). 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 constituted of GalA (71.8 and 75.6 g/100 g F3 and F′3, respectively) but had a higher amount of Rha than F2 and F′2 fractions. The GalA/Rha molar ratios of the F3 and F′3 (25 and 27, respectively) were higher than that of the F2 and F′2, indicating that the F3 and F′3 contained a higher proportion of rhamnogalacturonic regions than the F2 and F′2. The F3 and F′3 had a GalA/Rha molar ratio in agreement with that of the $P_H$. The DM of the F3 and F′3 were lower than for F2 and F′2 (52 and 58, respectively) and the DAc were still low (7 and 6, respectively). The $P_H$ of the two genotypes were very similar, regarding to the yields and compositions of the three first fractions. The fourth fraction was 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 constituted of GalA (71 g/100 g F4), whereas GalA represented only 35% of F′4. The DM of F4 and F′4 were, respectively, 18 and 38 and the DAc 2 and 4.

Table 4
Yields and compositions of the fractions purified by anion exchange chromatography of the PH extracted from genotypes RG822 and NV9392

| Genotype | Fraction | Yield[a] | Sugar composition[b] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Rha | Fuc | Ara | Xyl | Man | Gal | Glc | GalA | DM | DAc |
| RG822 | F1 | 29 | 0.3 | 0.3 | 27.6 | 0.8 | 0.4 | 3.3 | 0.7 | 1.2 | nd | nd |
| | F2 | 16 | 0.3 | 0.1 | 0.7 | 0.3 | 0.3 | 0.7 | 0.5 | 57.2 | 93 | 3 |
| | F3 | 32 | 2.2 | 0.2 | 1.6 | 1.0 | 0.2 | 2.2 | 0.6 | 63.8 | 52 | 7 |
| | F4 | 23 | 1.3 | 0.1 | 0.7 | 0.4 | 0.2 | 0.8 | 0.4 | 71.0 | 18 | 2 |
| NV9392 | F′1 | 32 | 0.4 | 0.1 | 38.9 | 1.0 | 0.7 | 6.1 | 2.1 | 1.7 | nd | nd |
| | F′2 | 19 | 0.3 | 0.1 | 0.7 | 0.4 | 0.3 | 0.8 | 0.5 | 64.5 | 82 | 4 |
| | F′3 | 45 | 2.1 | 0.2 | 1.6 | 0.8 | 0.2 | 2.1 | 0.8 | 67.8 | 58 | 6 |
| | F′4 | 4 | 1.4 | 0.0 | 0.9 | 0.7 | 0.6 | 1.1 | 0.9 | 35.0 | 38 | 4 |

nd, not determined.
[a] g/100 g $P_H$.
[b] g/100 g fraction.

## 4. Discussion

This study on the pectins of the fruit of the Japanese quince was carried out on two genotypes of *C. japonica*. A sequential extraction was performed: water and oxalate solubilised low amounts of pectins ($P_W + P_O = 6.1$ and 5.6 g/100 g AIS for genotypes RG822 and NV9392, respectively). These conditions were not degradative and pectin structure was preserved. Indeed, a very high and unusual intrinsic viscosity was measured for these pectins either with the Viscotek system or using an Ubbelhode capillary viscometer. Further investigation on the gelling properties of the $P_W$ and $P_O$ and on their fine structure would be interesting.

In contrast, dilute acid extracted a high quantity of pectins (26.5 and 23.7 g/100 g AIS for genotypes RG822 and NV9392). It represented 81% of the total quantity of extracted pectins. These extraction conditions were degradative and are known to break some covalent linkages, especially those involving Ara residues. Indeed, the quantity of Ara residues separated as neutral polysaccharides by anion exchange chromatography was very high, indicating that F1 and F′1 were mainly constituted of arabinans. The intrinsic viscosity of the $P_H$ was rather low compared to that of the $P_W$ or $P_O$ and may indicate that acidic conditions of extraction were degradative for the pectins.

The quince pectins contained GalA, galactose, arabinose, rhamnose, xylose and a little fucose. In the $P_W$ and $P_O$, Ara and Gal were present in similar amounts, whereas, the $P_H$ contained an increased proportion of Ara. The $P_H$ also had a lower GalA/Rha molar ratio (26 and 34 for RG822 and NV9392, respectively) than the $P_W$ (32 and 36) and the $P_O$ (44 and 66), indicating a higher proportion of rhamnogalacturonans in the $P_H$ than in the $P_W$ or $P_O$. This result was in agreement with those described for apple by Massiot et al. (1994) and for sugar beet by Fares et al. (2001).

The $P_H$ were constituted of four populations, the first one (F1 and F′1) being composed of arabinan, the second one of highly methylated homogalacturonans, the third one of rhamnogalacturonans and the fourth one differed according to the genotype studied.

Very few material was lost during the sequential extraction of pectins; indeed, 88.7 and 88.5% of the initial material were recovered from genotypes RG822 and NV9392, respectively. The recovery of each sugar during the extractions was high (from 70 to 100%), except for Ara which had a low recovery yield (57% for both genotypes), indicating that some arabinans were lost during pectin extraction.

The topology of the flesh tissue was observed by miscroscopy after each extraction step. This study showed that water or oxalate had a very limited effect on the topology of the flesh tissue, whereas dilute acid dramatically affected the structure of the tissue. However, it was not possible to determine whether the extracted pectins came from the primary cell-wall of the cells or from the middle lamella.

Finally, it was possible to extract 11.6 and 11.3 g pectins/100 g dry fruits and 1.4 and 1.3 g pectins/100 g fresh fruits of genotypes RG822 and NV9392, respectively. No significant difference was observed in the quantity or composition of the pectins extracted from the two genotypes. Only the proportions of the different families constituting the $P_H$ differed between the two genotypes as shown by their anion-exchange chromatograms. The quantities of pectins obtained in this work are higher than those obtained for Japanese quince by Golubev, Kolechik, and Rigavs (1990). Thakur et al. (1997) have reported apple (*Malus* spp.) to contain from 0.5 to 1.6 g pectins/100 g fresh apples. Japanese quince is thus an interesting source of pectins regarding to its pectin content. Further investigations should be done to better characterize their physicochemical properties and particularly their gelling properties.

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