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# Changes in the pectic substances of apples during development and postharvest ripening. Part 2: Analysis of the pectic fractions

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Pectic material was extracted from the alcohol-insoluble residues of apples during growth on the tree and at different stages of ripening during postharvest storage. Three pectic fractions, soluble in the chelating agent *trans*-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid (CDTA) in cold dilute sodium carbonate and in sodium carbonate at room temperature, were isolated. The galacturonic acid, neutral sugar and starch content of the fractions were determined as well as the degrees of methyl esterification and acetylation of the CDTA-soluble fraction. In addition, the molecular weight distribution was examined by gel filtration chromatography.

The high galacturonic acid content of the CDTA-soluble fraction was consistent with the polysaccharide being essentially linear and originating in the middle lamella. The sodium carbonate-soluble pectin had a higher rhamnose to galacturonic acid ratio, indicating that it was more highly branched. Galactose and arabinose residues were lost in the polymers from all three fractions during ripening, with the greatest loss occurring from the sodium carbonate-soluble fractions. At the same time, the pectins became less polydisperse and shifted towards higher molecular weights. It seems that during development there is a dynamic turnover with new pectic polymers, which contain fewer neutral sugars, but are of high molecular weight, appearing in the middle lamella and primary cell wall.

## INTRODUCTION

As reported in Part 1 (Fischer & Amadò, 1994), the major features of fruit ripening are flesh softening and textural changes. A degradation of the pectic backbone of the middle lamella and primary cell wall is usually suggested as the main cause of these changes, and an increase in soluble pectin and a loss of non-cellulosic neutral sugars have been reported for many ripening fruits (Gross & Sams, 1984).

The plant cell wall consists of several layers. The most external one is the middle lamella; it is the first one deposited at cell division and connects adjacent cells acting as a kind of cement between them. It is primarily composed of pectic substances and has a decisive influence on the texture of fruit tissue. The next layer, called the primary wall, is deposited while the cell is growing. It is rather thin and shows a higher degree of organisation than the middle lamella. Besides pectin it is composed of hemicelluloses and cellulose as well as some proteins.

One usually distinguishes between two main types of pectin according to their location in the cell wall matrix (Selvendran, 1985):

—The pectic polymers of the middle lamella are essentially linear with only short side-chains consisting of different neutral sugars, mostly arabinose and galactose, which are attached to the rhamnose (Rha) residues of the rhamnogalacturonan backbone. The pectins from the middle lamella possess a high degree of methyl esterification overall and the rhamnogalacturonan backbone is formed of regions of non-esterified residues interspersed with regions that are highly esterified and highly branched. The non-esterified regions are

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held together by ionic calcium bonds, and this pectin can be extracted by chelating agents which remove the calcium by complexation.

—The pectic polymers of the primary cell wall are more highly branched and the side-chains are longer. The rhamnogalacturonan backbone has a lower degree of methyl esterification.

To extract the middle lamella pectin the chelating agent *trans*-1,2-diaminocyclohexane-*N*,*N*,*N'*,*N'*-tetraacetic acid (CDTA) has proved effective (Selvendran *et al.*, 1985). Extraction with 0·05 M sodium carbonate at 1°C after CDTA gives good yields of more highly branched pectin originating in the primary cell wall. Extraction at this low temperature with addition of sodium borohydride minimises $\beta$-elimination. Subsequent extraction with the same extracting agent at room temperature solubilises more pectic substances. It has been suggested that non-esterified carboxylic groups of the galacturonic acid (GalUA) residues may be ester-linked to hydroxyl groups of the hemicellulosic sugars and these ester linkages would be saponified by the alkaline solvent, thus releasing the pectic polymers in solution (Selvendran *et al.*, 1985). However, as yet, there are no data to support the idea that the sodium carbonate-soluble fraction is covalently linked to the hemicelluloses. The remaining tissue is essentially free of pectin though some does remain in the cellulose residue even after hemicellulose extraction with 4 M sodium hydroxide.

In the first part we examined the changes occurring to pectic substances in the alcohol-insoluble residue (AIR) during growth and postharvest ripening of apples (Fischer & Amadò, 1994). We now report on the changes observed in the pectic polymers extracted from the AIR at various stages in the development and ripening of apples.

## MATERIALS AND METHODS

The methods for preparation of the AIR, for determination of galacturonic acid, starch and the neutral sugars as well as the degrees of methyl esterification and acetylation are as described in Part 1 (Fischer & Amadò, 1994). The only difference is that for neutral sugar analysis, the cell wall fractions (about 15 mg) were hydrolysed for 3 h at 100°C in 3 ml of 1 M sulphuric acid while stirring.

All the analyses, except for the extractions, which were carried out once, were performed in duplicate and the coefficients of variation were less than 5%.

### Extraction of the pectic material

Fractionation was carried out according to the method developed by Selvendran *et al.* (1985), which is summarised in Fig. 1.

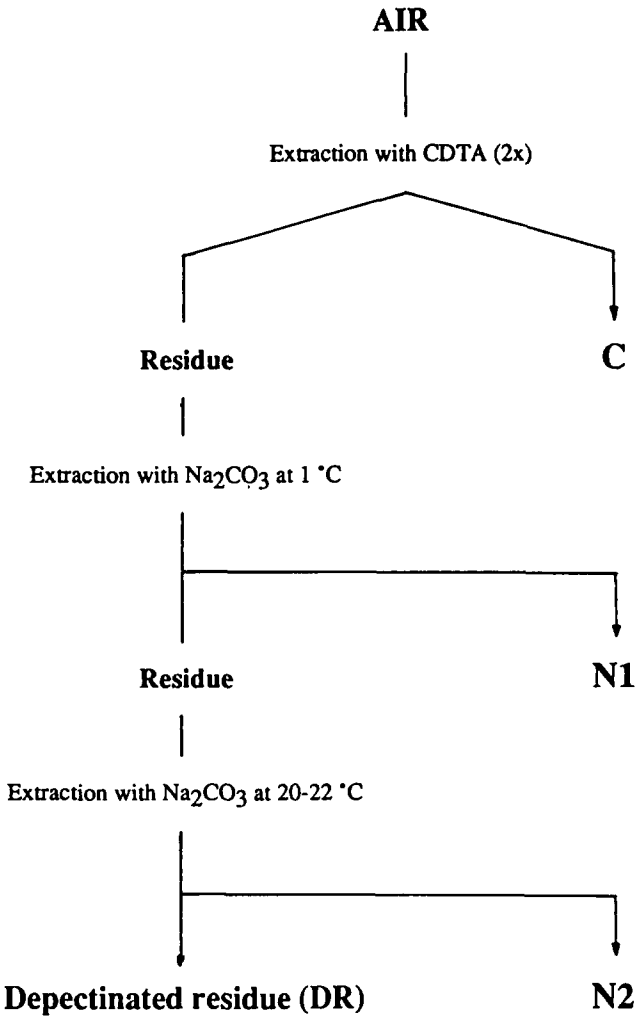


**Fig. 1.** Extraction scheme for the pectic cell wall fractions.

About 10 g of AIR was stirred with 1000 ml of 50 mM CDTA at pH 6·5 at 20 to 22°C for 6 h. The mixture was filtered on a G3 sintered glass filter funnel, and the residue was washed with distilled water. The residue was then further extracted with 1000 ml of CDTA for 2 h under the same conditions. The filtrate and washings from both extractions were combined, centrifuged at 10 000 *g* for 20 min (Sorvall RC 5B centrifuge, Du Pont Instruments, Wilmington, USA) and dialysed (Nadir, Hoechst, Wiesbaden, Germany; molecular weight cut-off: 5000) against several changes of distilled water. Dialysis was continued until the water in the container reached the conductivity of pure distilled water (about 1 week). After dialysis the extract was concentrated with a rotatory evaporator at 30°C under vacuum and freeze-dried (Edwards, Modulyo, Zivy & Cie, Oberwil, Switzerland). These two first steps yielded extract C.

The residue was then stirred for 16 h at 1°C with 1000 ml of a solution of 50 mM sodium carbonate and 20 mM sodium borohydride. The mixture was then

filtered on a G3 sintered glass filter funnel, and the residue was washed with distilled water. The filtrate and washings were brought to pH 5 with 2 M acetic acid, dialysed, concentrated and freeze-dried as above. This yielded extract N1.

The residue was then stirred for 3 h at 20 to 22°C with 1000 ml of a solution of 50 mM sodium carbonate and 20 mM sodium borohydride. After filtration the residue was washed with distilled water, dialysed and freeze-dried as above. This yielded the depectinated residue DR. The filtrate and washings were treated as above. This yielded extract N2.

All the extracts and the residue were stored at −20°C until needed.

### Gel filtration analysis

Gel filtration analysis (column 135 cm × 1·7 cm) was carried out using Sephacryl S-400 High Resolution gel (Pharmacia LKB, Uppsala, Sweden), eluted with a sodium acetate buffer (50 mM, pH 5·1) at a flow rate of 20 ml/h. A weight of sample corresponding to 3 mg of anhydrogalacturonic acid was dissolved in 2 ml of sodium acetate buffer (50 mM, pH 5·1) and applied to the column. For the samples containing starch, the samples were dialysed overnight against distilled water after enzymatic degradation of starch and were then applied onto the column.

Dextran standards were used for calibration. The exclusion ($V_0$) and total volume ($V_t$) of the system were 90 and 212 ml, respectively.

Fractions of 3 ml were collected and assayed for galacturonic acid and neutral sugars (Bailey, 1958) using an automated segmented flow analyser (Skalar Analytical, Breda, The Netherlands) by a method similar to that of Thibault (1979).

The segmented flow analyser system was calibrated with combined standards containing galacturonic acid in concentrations between 10 and 100 μg/ml and glucose in concentrations between 5 and 100 μg/ml. The total neutral sugars were thus evaluated compared with a glucose standard.

Where appropriate, depending on the shape of the peaks, selected fractions were pooled, freeze-dried and analysed for their neutral sugar content.

## RESULTS AND DISCUSSION

The changes in the pectic polymers were in fact studied for two apple varieties, Golden Delicious and Glockenapfel, for two seasons, and the same trends were observed in all cases. Only the values for the Golden Delicious apples during one season are presented here for the sake of simplicity. The data from another harvest will only be mentioned if it differs markedly. The complete data were collected in a PhD thesis (Fischer, 1993).

### 3.1 Yield of the fractions

Total recoveries of the individual fractions accounted for between 70 and 95% of the original cell wall material (Table 1). No trend towards an increase or a decrease in the yield of any fraction during development was noticeable. Only minor changes in the yields of the pectic fractions extracted from tomatoes (Gross, 1984), mangoes (Mitcham & McDonald, 1992) and nectarines (Dawson *et al.*, 1992) during ripening have also been reported. In kiwifruit, however, a decrease in fractions C and N was observed (Redgwell *et al.*, 1990).

### Composition of the pectic fractions and changes during development

The galacturonic acid and neutral sugar content of fractions C, N1 and N2 were quite different (Tables 2, 3 and 4). This can be related to increasing ratios of the neutral sugars to galacturonic acid in the C, N1 and N2

**Table 1. Yield (g/100 g) of the fractions after fractionation of the AIR of Golden Delicious apples during growth and ripening**

| Weeks | C | N1 | N2 | DR | Total |
|---|---|---|---|---|---|
| 0 | 16·8 | 12·4 | 7·0 | 52·7 | 88·9 |
| 3 | 17·6 | 12·2 | 2·2 | 63·7 | 95·7 |
| 6 | 13·8 | 10·8 | 5·0 | 49·9 | 79·5 |
| 9 | 23·1 | 7·2 | 2·1 | 38·1 | 70·5 |
| 12 | 16·3 | 12·1 | 3·3 | 56·5 | 88·2 |
| 15 | 15·8 | 11·5 | 4·2 | 56·5 | 88·0 |
| 18 | 17·6 | 15·7 | 5·0 | 56·5 | 94·8 |
| 21 | 13·8 | 12·0 | 5·4 | 55·8 | 87·0 |
| 24 | 14·6 | 11·3 | 5·4 | 67·3 | 98·6 |
| 27 | 16·4 | 11·5 | 4·9 | 60·6 | 93·4 |
| 30 | 16·5 | 14·8 | 1·7 | 62·5 | 95·5 |
| 33 | 15·8 | 12·8 | 3·2 | 57·7 | 89·5 |
| 36 | 12·7 | 10·9 | 3·4 | 58·4 | 85·4 |
| 38 | 15·3 | 12·1 | 4·3 | 57·8 | 89·5 |

**Table 2. Galacturonic acid and neutral sugar distribution (mol%) of fraction C from Golden Delicious apples during growth and ripening**

| Weeks | GalUA | Rha | Fuc | Ara | Xyl | Glu | Gal | Total (μg/mg) |
|---|---|---|---|---|---|---|---|---|
| 0 | 60·0 | 2·1 | 4·3 | 10·8 | 2·0 | 9·4 | 9·2 | 319 |
| 6 | 51·3 | n.d. | 3·9 | 8·3 | 1·7 | 23·9 | 11·0 | 345 |
| 12 | 61·4 | n.d. | 3·7 | 10·5 | 2·2 | 12·7 | 9·5 | 323 |
| 18 | 76·5 | 1·4 | 1·8 | 8·2 | 0·7 | 4·2 | 7·2 | 457 |
| 21 | 62·3 | 1·3 | 2·8 | 15·8 | 1·6 | 2·1 | 9·5 | 472 |
| 27 | 76·8 | 1·3 | 1·6 | 11·3 | 1·2 | 1·3 | 6·5 | 535 |
| 33 | 75·6 | 1·7 | 1·7 | 11·8 | 1·2 | 1·2 | 6·9 | 447 |
| 38 | 81·0 | 1·9 | 2·4 | 6·7 | 1·6 | 1·8 | 4·6 | 516 |

n.d. = not detected.

**Table 3. Galacturonic acid and neutral sugar distribution (mol%) of fraction N1 from Golden Delicious apples during growth and ripening**

| Weeks | GalUA | Rha | Fuc | Ara | Xyl | Glu | Gal | Total (μg/mg) |
|---|---|---|---|---|---|---|---|---|
| 0 | 35·7 | 3·9 | 3·2 | 28·9 | 0·8 | 3·0 | 23·7 | 472 |
| 6 | 60·5 | 1·9 | 1·5 | 18·7 | 0·7 | 0·2 | 16·5 | 989 |
| 12 | 64·2 | 1·9 | 1·4 | 19·5 | 0·9 | n.d. | 12·1 | 833 |
| 18 | 69·1 | 2·6 | 1·3 | 17·5 | 1·1 | 0·4 | 8·0 | 758 |
| 21 | 59·8 | 2·0 | 2·9 | 22·2 | 6·7 | 0·6 | 5·8 | 751 |
| 27 | 62·8 | 4·0 | 2·1 | 21·1 | 1·5 | n.d. | 8·5 | 560 |
| 33 | 62·9 | 2·8 | 1·1 | 23·9 | 2·1 | n.d. | 7·3 | 703 |
| 38 | 67·1 | 3·8 | 2·2 | 17·5 | 2·6 | 0·7 | 6·2 | 690 |

n.d. = not detected.

**Table 4. Galacturonic acid and neutral sugar distribution (mol%) of fraction N2 from Golden Delicious apples during growth and ripening**

| Weeks | GalUA | Rha | Fuc | Ara | Xyl | Glu | Gal | Total (μg/mg) |
|---|---|---|---|---|---|---|---|---|
| 0 | 15·7 | 3·2 | 1·9 | 36·9 | 1·1 | 1·9 | 39·2 | 837 |
| 6 | 21·0 | 1·9 | 1·7 | 31·8 | 1·5 | 6·4 | 35·7 | 972 |
| 12 | 26·1 | 2·9 | 1·5 | 35·5 | 2·2 | 2·1 | 29·7 | 877 |
| 18 | 45·6 | 3·3 | 1·1 | 29·7 | 2·1 | 0·6 | 17·6 | 805 |
| 21 | 37·5 | 2·3 | 1·6 | 37·1 | 2·6 | 0·6 | 16·0 | 890 |
| 27 | 31·7 | 2·7 | 1·1 | 40·6 | 3·5 | 1·6 | 18·8 | 731 |
| 33 | 43·7 | 2·8 | 1·2 | 32·5 | 3·3 | 0·9 | 13·2 | 648 |
| 38 | 50·1 | 4·7 | 2·0 | 26·1 | 3·9 | 0·7 | 12·5 | 685 |

fractions, respectively. The neutral sugar and galacturonic acid contents are expressed as molar ratios, the sum of both being taken as 100. The values for glucose have been corrected for the starch content, and the last column presents the recovery of uronic acid and neutral sugars in the starch-free fractions. The representation in molar percentage yields the same results as when using the weights of the various components in the material, but does not take into account the contribution of the extra constituents such as the proteins, water and ash.

Many of the fractions contained traces of mannose. This sugar has been detected in the pectic substances of apples by Voragen *et al.* (1983), Aspinall & Fanous (1984), Stevens & Selvendran (1984) and Renard *et al.* (1990), but not by Knee (1973) and de Vries *et al.* (1981). Since the amounts are low (0·5 mol%), this sugar is not reported in the tables.

During growth and ripening there was a general increase in galacturonic acid and a decrease in neutral sugars in all pectic fractions, particularly galactose. The loss of non-cellulosic neutral sugars seems to be a general feature of ripening fruit.

*Fraction C*

Fraction C contained the lowest proportion of neutral sugars of all three pectic fractions (Table 2). It also had the lowest Rha:GalUA ratio (about 1:50) and since rhamnose is the point of attachment for the side-chains, this is consistent with the assumption that the major part of the chelator-soluble pectin is a homogalacturonan-type polymer which originates in the middle lamella (Selvendran, 1985). Xylose and glucose were present in fraction C despite the fact that they are major components of xyloglucans. The presence of a neutral polysaccharide containing high proportions of glucose and xylose in the chelator-soluble fraction has been reported before (de Vries *et al.*, 1981; Stevens & Selvendran, 1984; Renard *et al.*, 1990). The origin of this polymer is unknown, but a recent report has demonstrated that not all the xyloglucan is hydrogen-bonded to the cellulose fibrils (Edelmann & Fry, 1992). However, the major part of the xylose present in the AIR was recovered in the hemicellulose fraction after fractionation of the DR (data not published). Arabinose was the predominant neutral sugar although in the unripe fruit it was not much more abundant than galactose. Rhamnose was not always detected in fraction C at the early stages of growth. It is known that Rha is often underestimated when Saeman hydrolysis is used since uronic acid glycosyl linkages, GalUA-α-(1 → 2)-Rha, for example, are relatively acid-resistant (Fry, 1988; De Ruiter *et al.*, 1992). Because the Rha content in these fractions is low, it could lie below the limit of detection.

In the unripe apples, CDTA solubilised about 22% of the GalUA present in the AIR (Fischer & Amadò, 1994). This value stayed relatively stable until harvest (12 weeks); it then increased slightly and was 28% in the ripe fruit. The GalUA content of this fraction increased by about 30% from the unripe to the senescent fruit (Table 2). Fraction C exhibited the smallest increase in GalUA of all the pectic extracts.

About half of the initial galactose from fraction C was lost in the senescent apples; the decrease was linear over the experimental time span (Table 2; Fig. 2). Arabinose stayed relatively constant at first, it increased suddenly after 20 weeks, then declined, but not as much as galactose. The relatively constant behaviour of these sugars during growth and the first few weeks of storage points to a metabolism where synthesis of the middle lamella pectin is still continuing despite the fact that there is already an overall loss in neutral sugars. Synthesis of cell wall material after cell expansion had ceased has been reported for tomatoes (Mitcham *et al.*, 1989). In the ripe to senescent fruit, the losses of galactose and arabinose seemed to follow a similar trend which could mean that they both come from the same polymers, and pectin degradation in the middle lamella may well occur via the loss and degradation of arabinogalactan side-chains and the concurrent degradation of arabinan and galactan side-chains.

Despite the fact that no clear shift towards either a lower or a higher average molecular weight was observed during gel filtration of fraction C, the

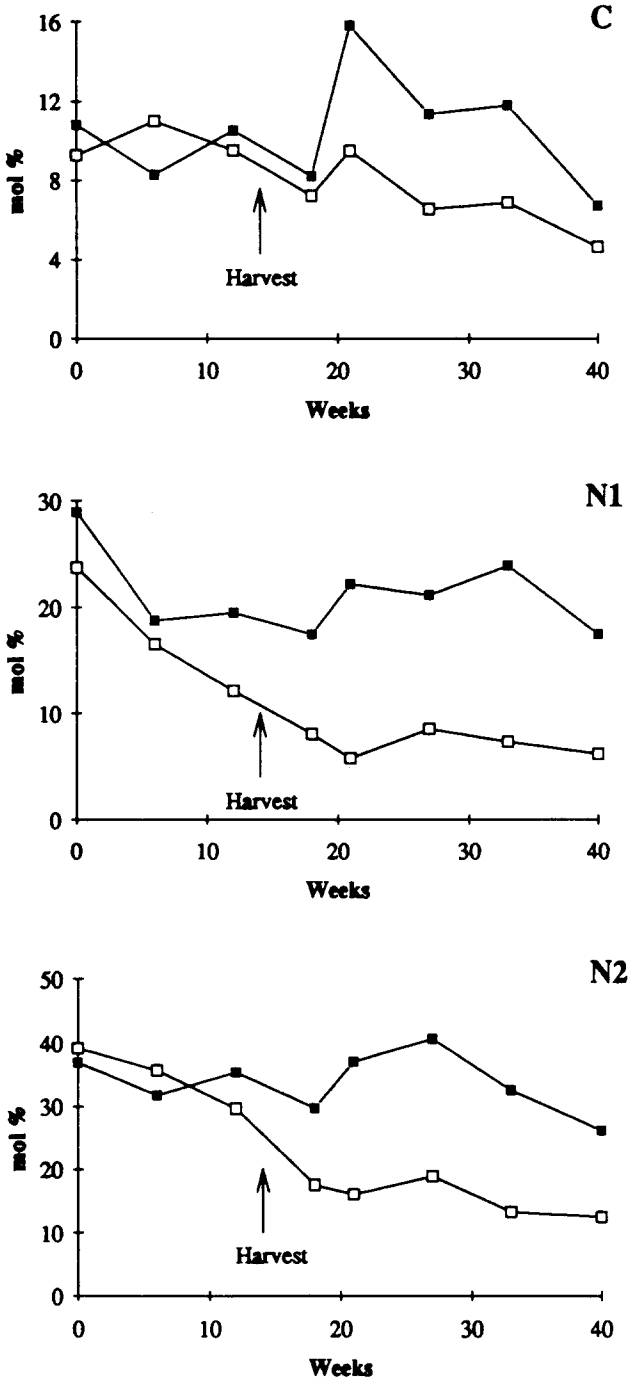


**Fig. 2.** Arabinose (■) and galactose (□) content of the pectic fractions of Golden Delicious apples during growth and ripening.

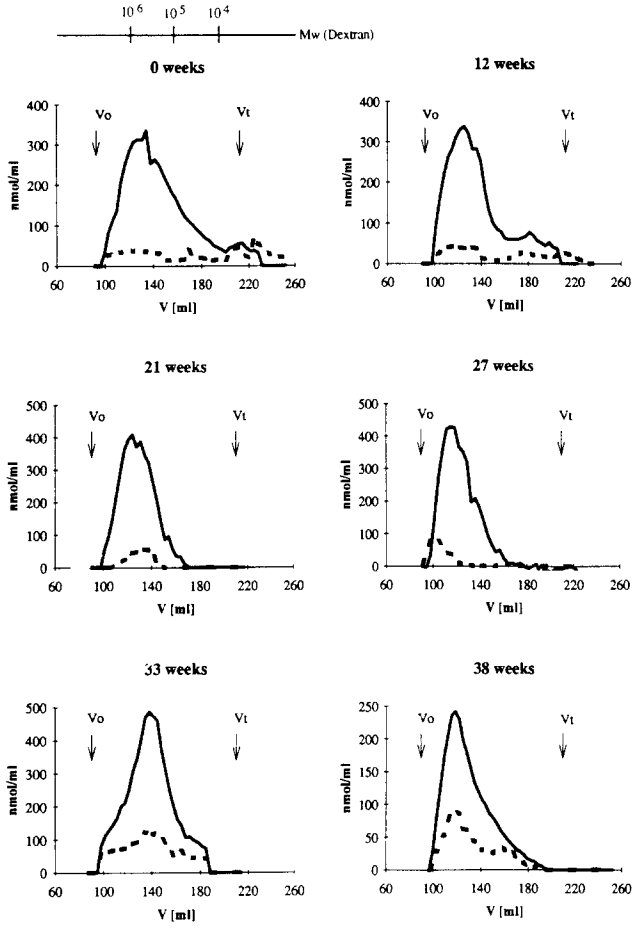


**Fig. 3.** Gel filtration patterns of fraction C of Golden Delicious apples at different stages of development. (Dashed line: neutral sugars; solid line: GalUA.)

appearance of the chromatograms did change with time (Fig. 3). The elution volume being dependent on the hydrodynamic volume, this indicates a change in the shape or the size of the polymers (Kravtchenko *et al.*, 1992). In the unripe fruit a broad molecular weight range was observed, whereas in the ripe and senescent fruit the peak was concentrated over a narrower, but equally high molecular weight range. This points to less polydispersity of the polymers in the senescent fruit. In the unripe fruit, the neutral sugar analysis of the pooled fractions showed that arabinose and galactose were present in similar amounts in the high molecular weight range, whereas polymers containing more galactose than arabinose were found in the lower molecular weight range. In the ripe fruit, arabinose predominates over galactose in the high molecular weight polymers. This is consistent with the observation of a slight accumulation of arabinose during growth, whereas galactose decreased steadily from the beginning.

In fruits which contain the enzyme *endo*-polygalacturonase (*endo*-PG), such as tomatoes (Huber, 1983*a*,*b*) and pears (Yoshioka *et al.*, 1992), a decrease in high molecular weight chelator-soluble pectins and a concomitant increase in low molecular weight polymers has been observed. In apples (Yoshioka *et al.*, 1992) where *endo*-PG activity is low (Wu *et al.*, 1993) or strawberries (Huber, 1984), which do not contain this enzyme, no substantial change in the molecular weight distribution of the chelator-soluble fraction has been seen. In melon, however, in which no *endo*-PG activity has been found, ripening was accompanied by a decrease in the molecular weight of the chelator-soluble

pectin (McCollum *et al.*, 1989). This raises once again the question of the complex relationships between solubilisation, depolymerisation and *endo*-PG activity. It seems, nevertheless, to validate the theory that depolymerisation is not essential to solubilisation (Giovannoni *et al.*, 1989).

*Fraction N1*

Fraction N1 had a slightly higher neutral sugar content than C, with arabinose being the main sugar once again (Table 3). The Rha:GalUA ratio was higher, about 1:25, which points to the presence of more highly branched polymers as predicted for pectins from the primary cell wall.

There also was an increase in the GalUA solubilised from the AIR (15–25%) (Fischer & Amadò, 1994). In this fraction the increase in the galacturonic acid content was larger than in fraction C (88%) (Table 3).

The galactose and arabinose contents decreased in this fraction, about 75% of the initial galactose being lost in the senescent fruit (Table 3; Fig. 2). The greatest loss occurred during the first 20 weeks, after which the decline was very slow. This is the opposite of what was observed in the chelator-soluble fraction where the losses mainly occurred in the ripe fruit. The decrease in the arabinose content was lower than that of galactose. The losses of these sugars followed different patterns and seemed independent of each other, which could signify that the degradation of pectin occurs via two different steps. First, there could be detachment of galactan side-chains which would lead to the appearance of a new kind of pectin containing more arabinogalactan or arabinan side-chains, which would then be lost during senescence. The loss of galactan side-chains has already been reported for apples (Jarvis, 1984) and kiwifruits (Redgwell *et al.*, 1992).

Xylose increased markedly in this fraction and could result from the solubilisation of hemicellulosic polymers. The fact that no concurrent increase in glucose was observed suggests that the hemicellulose involved was not xyloglucan, but may have been a xylan. Such a hemicellulose has been shown to occur in the cell wall of kiwifruit (Redgwell *et al.*, 1988). Alternatively, the change could be in the xylose residues attached directly to the rhamnogalacturonic backbone and would correspond to modification of the existing pectin.

The gel filtration chromatograms of fraction N1 are shown in Fig. 4. Again, there was hardly any molecular weight shift, but a change in the composition of the fractions was observed. In unripe fruit, two different populations were apparent with polymers with a lot of uronic acid in the intermediate molecular weight range and polymers containing a lower GalUA to neutral sugar ratio in the high molecular weight range. During ripening there was an accumulation of polymers containing large amounts of neutral sugars in the high molecular weight range while the intensity of the peak

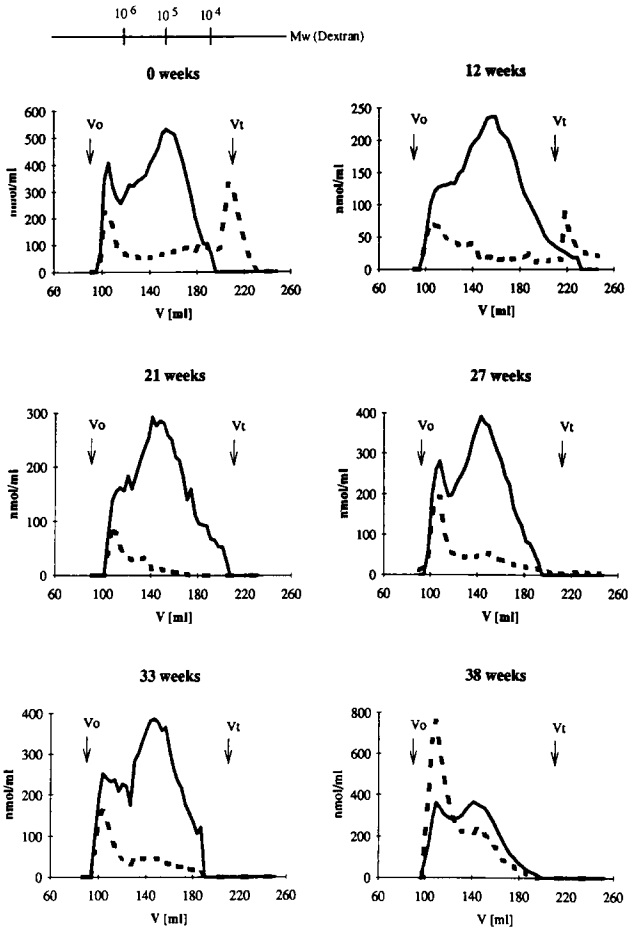


**Fig. 4.** Gel filtration patterns of fraction N1 of Golden Delicious apples at different stages of development. (Dashed line: neutral sugars; solid line: GalUA.)

in the intermediate range decreased, although two distinct populations remained. The high molecular weight pectins which contain most of the neutral sugars probably corresponded to highly branched 'hairy' regions.

Analysis of the pooled fractions showed more arabinose than galactose in the high molecular weight region, but more galactose than arabinose in the low molecular weight region. During development, the ratio of arabinose to galactose increased as expected, since more galactose was lost during ripening. The glucose content of the neutral sugar peak near the total volume in the unripe samples probably results from incomplete removal of partially degraded starch.

*Fraction N2*

Fraction N2 contained the highest proportion of neutral sugars, particularly arabinose (Table 4). The Rha:GalUA ratio was very high, about 1:10, showing that these pectic polymers are the most highly branched. Xylose was also more abundant than in the other fractions. The presence in the alkali-soluble fractions of a highly branched rhamnogalacturonan with xylose resi-

dues or side-chains attached directly to the galacturonic acid residues has been reported (Renard, 1989).

In fraction N2 the amount of GalUA solubilised from the AIR remained around 7–10% and no clear trend was discernible (Fischer & Amadò, 1994). Despite the fact that there was no change in the amount of GalUA extracted from AIR, fraction N2 presented the largest increase in GalUA relative to the weight of the fraction during growth and ripening, since the GalUA content more than tripled. The apparent contradiction between the amount of GalUA solubilised from the AIR and the GalUA content stems from the large loss in neutral sugars in this fraction.

The galactose and arabinose content of fraction N2 decreased overall (Table 4). Most of the galactose residues were lost during the first 20 weeks just as in fraction N1. Arabinose increased slightly before declining during the last 10 weeks. This may be due to the synthesis of new branched pectin during growth and at the beginning of ripening. Loss of arabinose residues only occurred after the onset of senescence. Here, as in fraction N1, the losses of galactose and arabinose residues did not occur in parallel, also supporting a two-stage mechanism. The loss of a galactan seemed to be the principal cause of pectin degradation and it preceded tissue softening, while senescence was associated with the additional loss of arabinose residues. The xylose content also rose markedly in fraction N2.

The gel filtration chromatograms of fraction N2 (Fig. 5) also showed a concentration of the polymers in the high molecular weight region during ripening. In the unripe apples, the polymers were more polydisperse.

Analysis of the pooled fractions showed that the high molecular weight polymers were generally also richer in arabinose than the low molecular weight ones. During ripening the ratio of arabinose to galactose showed an increasing tendency similar to that in fraction N1, which is logical since more galactose than arabinose was lost. The losses seemed to be greatest in the low molecular weight regions. As in fraction N1, two different populations were visible: one with high molecular weight polymers which were rich in neutral sugars and one of lower molecular weight with a lower neutral sugar content. Only at week 0 was a large neutral sugar peak apparent at the bottom end of the range. This peak was shown to consist mainly of galactose and disappeared during development, which could be linked to the loss of galactan side-chains mentioned earlier. In this fraction as well the high molecular weight fractions corresponded to highly branched 'hairy' regions.

## Starch content

The starch content decreased until harvest (Table 5). A few weeks after harvest (week 14), the amount of glucose determined by derivatisation and gas chromatography was low (less than 20 μg/mg cell wall material), which points to the fact that the starch has been almost totally degraded.

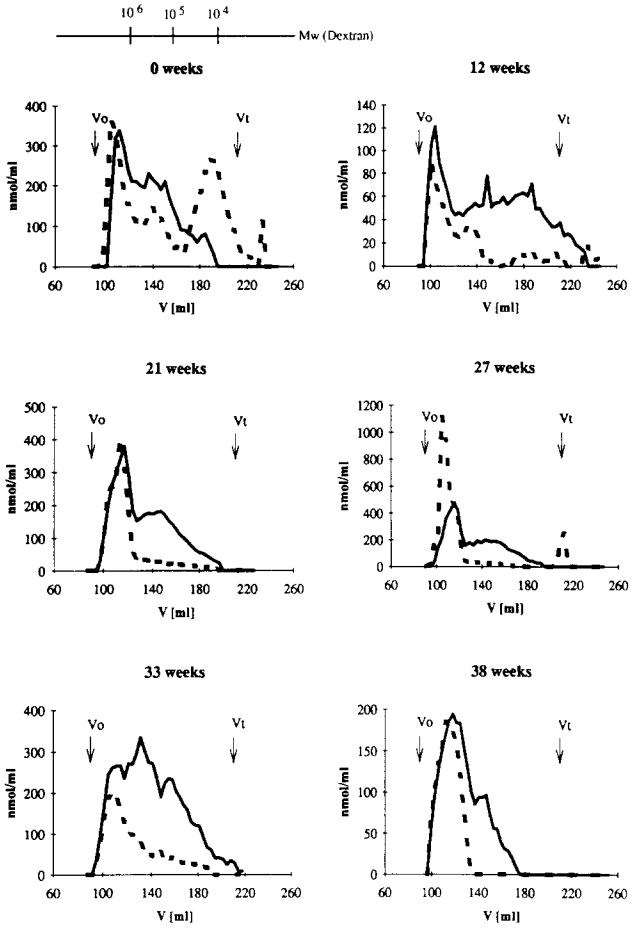


**Fig. 5.** Gel filtration patterns of fraction N2 of Golden Delicious apples at different stages of development. (Dashed line: neutral sugars; solid line: GalUA.)

**Table 5. Starch content (g/100 g) of the pectic fractions from Golden Delicious apples during growth**

| Weeks | C | N1 | N2 |
|---|---|---|---|
| 0 | 19·5 | 16·6 | 14·3 |
| 6 | 21·5 | 13·1 | 22·4 |
| 12 | 15·3 | 2·6 | 4·2 |

## Degrees of methylation (DM) and acetylation (DA)

Only the DM and DA of the chelator-soluble fractions were measured since the alkali-soluble fractions N1 and N2 would be totally or partially saponified by the conditions of extraction. These values are reported in Table 6 and are based on the GalUA content of the fractions.

The DM of the CDTA-soluble pectin was slightly lower than that of the AIR, but it nevertheless reached

**Table 6. Degrees of methyl esterification and acetylation of fraction C from Golden Delicious apples during growth and ripening**

| Weeks | DM (%) | DA (%) |
|---|---|---|
| 0 | 51·4 | 5·5 |
| 3 | 57·9 | 4·6 |
| 6 | 60·2 | 5·2 |
| 9 | 76·5 | 6·9 |
| 12 | 69·7 | 4·6 |
| 15 | 97·5 | 5·2 |
| 18 | 60·5 | 3·4 |
| 21 | 95·7 | 4·0 |
| 24 | 78·0 | 3·8 |
| 27 | 80·2 | 6·1 |
| 30 | 95·2 | 4·7 |
| 33 | 84·3 | 3·9 |
| 36 | 81·6 | 3·9 |
| 38 | 63·6 | 3·9 |

values close to 100% in the pectin from the ripe fruit. As for the AIR, there was no noticeable change to the DM through development and ripening (Fischer & Amadò, 1994).

The role of the DM during ripening of fruits and vegetables has often been questioned since it is not clear whether an increase or a decrease can best explain the softening and pectin solubilisation observed.

A high level of methyl esterification leads to weak ionic binding of the pectic polymers with divalent calcium ions and would thus promote less cohesion of the middle lamella pectin. Knee (1978*a*,*b*) observed an increase in the DM of water-soluble pectin despite no change in the overall cell wall methyl esterification. He suggested that during ripening a new highly esterified pectin replaced the initial middle lamella pectin, which was degraded during this process.

On the other hand, in fruit where *endo*-PG is present, a decrease in the DM would favour the effectiveness of the enzyme since *endo*-PG only cleaves the bonds between unesterified GalUA residues. Yet no clear correlation between PG and pectinmethylesterase (PME) activities has been observed so far (Huber, 1983*a*), so for the moment no conclusive links between the DM, PME activity and fruit ripening exist.

Renard & Thibault (1993) have challenged the hypothesis that the CDTA-soluble fraction consists only of the middle lamella pectin, arguing that the quantities of pectin extracted by CDTA are too high to originate solely from the middle lamella. Also, the high DM of this pectic fraction does not favour the formation of stable ionic bonds with divalent calcium ions according to the 'egg box' model. Certainly the DM values found in this study are very high and it is tempting to suggest that while fraction C does contain the middle lamella pectin, it also contains appreciable amounts of highly esterified unbranched pectin which could come from the primary cell wall.

The DA was much lower than that of the AIR. It was decreased steadily during growth and ripening. The role of the DA in pectin is not well understood, although it certainly affects the cohesive properties of the pectic polymers. It is known that sugar beet pectins have poor gelling properties due to a high DA (Rombouts & Thibault, 1986), but the link between observations made *in vitro* and *in vivo* behaviour is speculative.

## CONCLUSIONS

A loss of galactose residues from the cell wall during ripening is a common feature in many fruits studied. In some fruits, but not all, a depolymerisation of the pectic polymers has also been observed. In this study, a marked loss of galactose was observed, but there was no lowering of the average molecular weight. This supports the theory that depolymerisation is not necessary for pectin solubilisation. The results seem to indicate that during growth and ripening there is a constant turnover of the cell wall polymers where a loss in sugar residues is usually compensated by *de novo* synthesis of cell wall polymers with a lower degree of branching, but nevertheless a high molecular weight. There is thus no marked loss in cell wall material, which could be why no net decrease in the weight of the cell wall fractions extracted was observed.

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