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# Isolation, purification and characterization of neutral polysaccharides from extracted apple juices

**Frank Will & Helmut Dietrich**

*Forschungsanstalt Geisenheim, Fachgebiet Weinanalytik und Getrankeforschung, PO Box 1154, W-6222 Geisenheim, FRG*

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Using membrane processes, the pectic material of an extracted apple juice was isolated, decolorized and degraded with purified pectin esterase (EC 3.1.1.11) and *endo*-polygalacturonase (EC 3.2.1.15). Separation of the resulting polysaccharides by anion-exchange-chromatography yielded two neutral and one acidic fractions. Enzymic hydrolysis with purified arabinofuranosidase, *endo*-α-1,5-arabanase and partial acid hydrolysis in combination with methylation analysis revealed linear α-L-1,5-arabans, highly branched type II-arabinogalactans and rhamnogalacturonans with a high content of neutral sugars, which could not be further degraded by purified pectinases. The isolated polymers are involved in clarification and stabilization problems of apple juice or concentrate.

## INTRODUCTION

Pectic substances in fruit juices have their origin in the middle lamella or the primary plant cell wall. During pressing or extraction they pass over into the juice. The amount of the carbohydrate colloids in the juice depends on the treatment of the apple mash. High shear stress in modern belt presses followed by water extraction of the pomace leads to increasing contents of pectic polysaccharides. Clarification will become more difficult, the higher the content of colloidally dissolved substances in the juice.

Many authors worked with apple cell wall material. The major part of these investigations are concerned with the AIR (Alcohol Insoluble Residue) of the comminuted apple mash. Voragen *et al.* (1983) fractionated the AIR of apples and found polymer substances like pectin, hemicellulose, cellulose, lignin and protein. DeVries *et al.* (1982, 1983) divided the pectin molecule into homogalacturonan (smooth) regions and rhamnogalacturonan (hairy) regions. The hairy regions carried side chains consisting mainly of arabinogalactans. Saulnier *et al.* (1988) subdivided the hairy regions of grape pectins into type II-arabinogalactans (hairy region A), which are associated with the homogalacturonan backbone. The hairy regions B are consisting of araban side chains, attached to the rhamnogalacturonan backbone of the pectin molecule.

Some authors worked on the cell wall material passing into the juice during pressing and extracting processes. Rouau and Thibault (1984) isolated apple juice pectin by ethanol precipitation. The pectic substances showed a low content of neutral sugars (6·8%) with equal amounts of arabinose and galactose. Kauschus and Thier (1985) found a pectin content of 200–400 mg/liter in freshly pressed apple juices. The isolated polymers consisted mainly of neutral sugars.

Schols *et al.* (1991) analysed the composition of the high molecular weight compounds in apple juices, obtained by straight pressing, pulp enzyming or liquefaction and subsequent clarification by conventional procedures or ultrafiltration. There was a strong influence of the processing method and enzyme preparation on the amount of colloid material in the juice, which differed by a factor of more than 20. The content of polysaccharides solubilized in the juices increased when enzymes were used. Ultrafiltration removed 50%, 50–65% and 80–90% of the polymers from the pressed juices, pulp enzyming and liquefaction, respectively. The distinct fouling of the ultrafiltration membranes was due to the presence of the polymers solubilized by the liquefaction process.

Clarification of apple juice is achieved by degradation of juice pectin with pectolytic enzyme preparations, followed by fining and filtration. In many cases the clarification process fails, and the juice remains hazy.

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The purpose of the present work was therefore to isolate, purify and characterize pectic polysaccharides from extracted apple juice in order to improve juice clarification and filtration.

## EXPERIMENTAL

### Apple juice

The apple juice was obtained from a German apple processing plant: it was derived from belt pressing combined with water extraction of cider apples. Only the water-extracted juice was used for the investigations. Its dry matter content was 5 Brix; before sampling it was concentrated to single juice strength (13 Brix). Neither in the mash nor in the juice stage were enzymes added.

### Isolation of the pectic material

The juice (300 liters) was separated (Westfalia CSA 1) and cross-flow microfiltered (Memcor Ltd, pore size 0·2 $\mu$m), the resulting MF-permeate was ultrafiltered (Sartorius, cut off 10 000 Daltons). The UF-retentate was concentrated, diafiltered, decolorized and freeze dried according to Wucherpfennig and Dietrich (1983).

### Enzymic degradation

The pectic material was degraded by purified pectinesterase (PE, EC 3.1.1.11), *endo*-polygalacturonase (PG, EC 3.2.1.15), arabinofuranosidase (EC 3.2.1.55) and *endo*-$\alpha$-1,5-arabanase (EC 3.2.1.99) isolated from technical enzyme preparations (Schopplein, 1989). The purified enzymes showed no significant side activities. Enzymic reactions were carried out in 0·05 M acetate buffer pH 5·0 at 40°C for 3 days: the concentration of the polysaccharides was 1%. After denaturation, the mixture was membrane-filtered (0·45 $\mu$m), and the filtrate was desalted by gel chromatography on Sephadex G 25 (20 cm × 5 cm, Pharmacia). The high molecular weight peak was collected and freeze dried.

### Anion exchange chromatography

The native juice pectin was degraded by purified PE/PG, and 350 mg quantities of depectinized material were dissolved in 20 ml water by stirring overnight. The solution was membrane-filtered (0·45 $\mu$m) and applied to a 15 × 2·2 cm column of DEAE sepharose CL-6B (Pharmacia) in the chloride form. Ten-milliliter fractions were eluted (100 ml/h) from the column by a step gradient of 120-ml portions of water and NaCl buffers of 50 m mol liter$^{-1}$ and 500 m mol liter$^{-1}$. Neutral and acidic sugar contents were measured by the orcinol (Svennerholm, 1956) and the *m*-phenylphenol assay (Blumenkrantz & Asboe-Hansen, 1973). Sugar-containing fractions forming a peak were pooled, desalted on Sephadex G 25 and freeze dried.

### Molecular weight distribution

The average molecular weights of the polymers were determined by FPLC on a Superose 12 column (30 × 1 cm, Pharmacia), calibrated with a dextrane standard kit (T 10–T 250, Pharmacia). The pump was a Pharmacia P 500 with a flow rate of 24 ml/h, the eluent was 0·2 M NaCl. Detection was achieved by a Waters R 401 differential refractometer: additionally fractions of 0·5 ml were collected and analysed for uronic acids with *m*-phenylphenol (Blumenkrantz & Asboe-Hansen, 1973).

### Sugar composition

Neutral sugars were determined after sulfuric acid-hydrolysis (Saeman *et al.*, 1945) and conversion to their alditol acetates (Blakeney *et al.*, 1983). The alditol acetates were separated by GLC on a fused-silica capillary column (DB 225, 15 m × 0·32 mm, J&W Scientific) with on-column injection at 60°C. Myo-inositol was used as the internal standard. Galacturonic acid was measured with the *m*-phenylphenol-assay.

### Linkage study by permethylation

Methylation analysis was performed with butyllithium and methyl iodide in dimethyl sulfoxide (Kvernheim, 1987). The per-*O*-methylated polysaccharides were hydrolysed with 2 M trifluoroacetic acid and derivatized to their alditol acetates according to Harris *et al.* (1984). The partially methylated alditol acetates were separated by GLC on a fused-silica capillary column (DB 225 30 m × 0·32 mm, J&W Scientific) after on-column injection at 50°C. The resulting peak areas were corrected by their effective carbon response factors (Sweet *et al.*, 1975). Identification of the peaks was confirmed by mass spectrometry on a Carlo Erba QMD 1000 mass spectrometer.

### Partial acid hydrolysis

The samples were dissolved in 0·1 M TFA and heated at 100°C for 1 h. The acid was removed in a rotary evaporator: the residue was dissolved in water and separated in a high and low molecular fraction by size exclusion chromatography on Sephadex G 25 (25 cm × 2·5 cm).

### $^{13}$C-NMR-spectroscopy

Selected samples were dissolved in $D_2O$: spectra were recorded at 75·46 MHz using a Varian spectrometer.

## RESULTS

### Isolation of the pectic material

The juice had been obtained from cider fruit firstly comminuted in a hammer mill, then pressed on a belt press and finally extracted. The colloid content of the single strength juice, extracted with hot water, was 2500 mg/liter of juice. In comparison, gently pressed apple juices derived from rack and cloth presses have colloid contents of about 400 mg/liter. For further decolorization, purification and removal of insoluble material, the raw juice was cross-flow microfiltered. The clear permeate from the microfiltration was subjected to ultrafiltration (molecular cut off 10 000 Daltons). Adhering phenolic compounds were removed during the diafiltration with citrate buffer and water. The combined membrane processes of micro- and ultrafiltration resulted in colorless and completely soluble polysaccharides. The colloid content of the MF-permeate was 160 mg/liter. Since it was prepared by micro- and ultrafiltration, it was called MF/UF.

### Fractionation

The pectinases achieved only a 60–70% degradation of the colloids. The remaining polysaccharides called PE/PG were further fractionated by anion exchange chromatography, yielding three different fractions named as shown in Fig. 1. The fractions $-H_2O$ and $-50$ mM NaCl were neutral and accounted for, respectively, 8 and 60% of PE/PG: the fraction $-500$ mM NaCl was an acidic polymer accounting for 32% of total PE/PG.

### Molecular weight distributions

Figure 2 shows the molecular weight distributions of the native juice pectin and the PE/PG-degraded sample. The native polymer, consisting mainly of galacturonic acid, eluted in the void volume of the column and indicated molecular weights >250 000

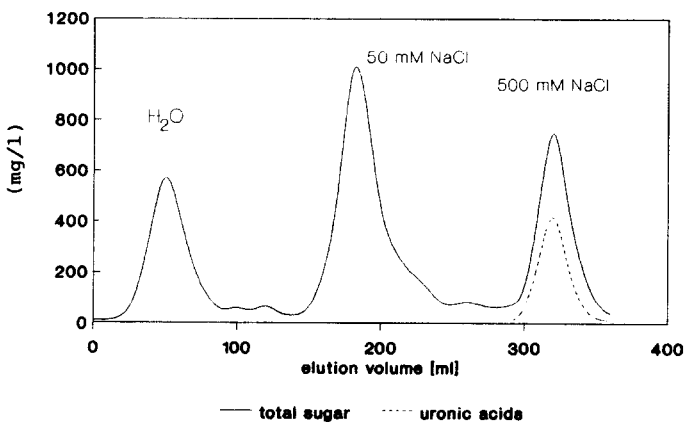


**Fig. 1.** DEAE-fractionation of sample PE/PG.

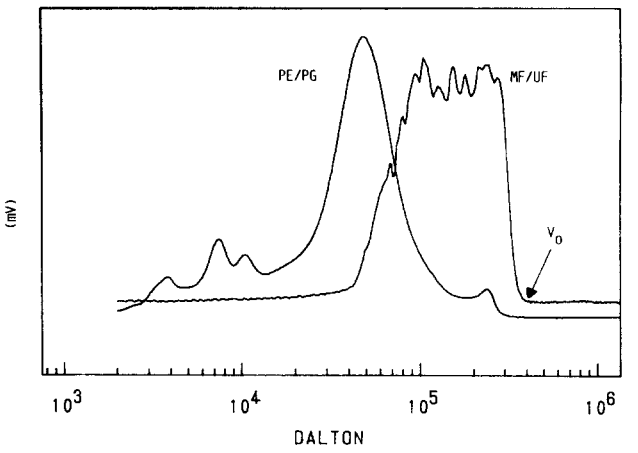


**Fig. 2.** Molecular weight distribution of MF/UF and PE/PG.

Daltons. The sample PE/PG consisted largely of neutral sugars: its molecular distribution shows a main peak at 50 000 Daltons, a peak at 250 000 and several fragments below 10 000 Daltons. As a result of this, a mixture of different colloids remained after PE/PG-degradation.

The molecular weight distributions of the DEAE-fractions are shown in Fig. 3. The water- and 50 mM-fraction coeluted except of a peak in the lower molecular range of $-H_2O$ near 10 000 Daltons (LM). The main peaks with maxima at 50 000 Daltons eluted nearly identically. The acidic fraction $-500$ mM shows higher molecular weights and a more complex distribution with acidic polymers in the void volume of the Superose 12 column and in the range near 8000 Daltons (hatched areas). Neutral peaks appeared at 100 000, 20 000 and 10 000 Daltons. Unlike the neutral 50 mM-fraction, $-$DEAE 500 mM is an heterogeneous mixture of different polysaccharides.

### Monomer composition

Table 1 shows the results of the sugar analysis of native and PE/PG-degraded colloids. The native juice pectin contained only 49% galacturonic acid. The main neutral sugars were arabinose and galactose: they accounted for 33·5% in the ratio 0·66. Further separation of the enzymatically degraded sample PE/PG by anion-exchange chromatography resulted in two neutral and one acidic polymer. The neutral polymers consisted mainly of arabinose and galactose in different ratios (see Table 1 for sugar composition). Further sugars found were rhamnose and glucose, accounting for 6·6 and 11·7%, respectively, of the $H_2O$ fraction. With the 500 mM ionic strength a pectin fragment with 11·2% galacturonic acid could be eluted from the anion exchange column. In no case was the yield of the sugars 100%. This is due to hydrolysis problems and impurities in the polysaccharides, particularly phenolic substances and protein (Will & Dietrich, 1990).

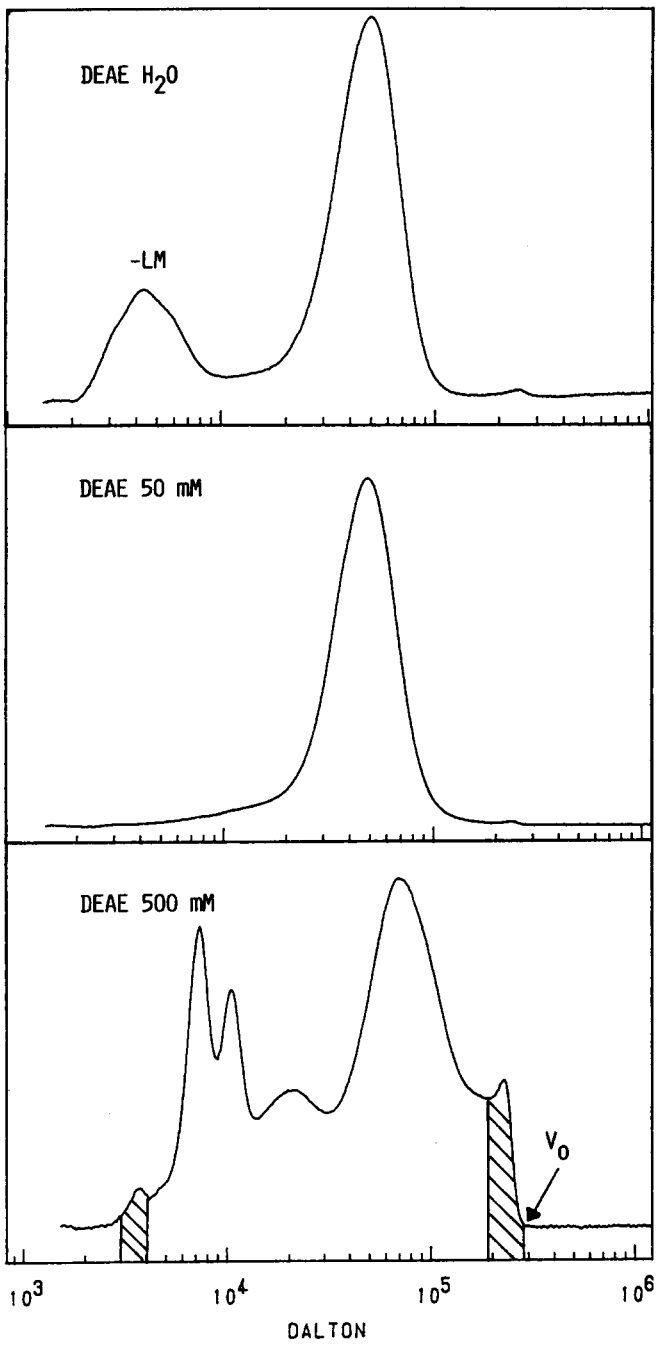


**Fig. 3.** Molecular weight distributions of the DEAE-fractions.

With regard to the monomer composition of the different colloids, arabinose and galactose dominated in varying ratios. After treatment of the native juice pectin with purified pectinases, the content of galacturonic acid decreased drastically to 15% in PE/PG. There was an enrichment of xylose and glucose in the DEAE fraction $-H_2O$, indicating xyloglucan from the apple cell wall. From the sharp molecular weight distribution and the results of the sugar analysis it could be concluded, that fraction −50 mM consisted of a homogeneous arabinogalactan. Fraction −500 mM NaCl had the lowest yield of neutral sugars: it accounted for 57·8%. The contents of galacturonic acid (11·2%) and rhamnose (6·7%) together with the high values for arabinose (24·0%) showed that this pectic fragment derived from the rhamnogalacturonan zones of the pectin molecule. Mannose could not be detected in any fraction.

**Linkage study of the arabinogalactan**

Methylation analysis of the neutral 50 mM fraction revealed a type II arabinogalactan with β-1,3-galactan main chains, branched in position 6 with β-1,6-linked galactan side chains. Table 2 shows the distribution of the methyl ethers (>1 mol%) before and after selective cleavage with purified arabinosidase and *endo*-1,5-arabanase (see Table 1 for alditol acetates). In respect to the arabinogalactan the data indicate that more than 70% of the present arabinose were in a terminal, non-reducing position: the remaining arabinose was 1,5-linked. The tremendous breakdown of the predominant galactose methyl ether 24-$Me_2$-gal and the large increase of 234-$Me_3$-gal after the action of the arabinosidase show that the terminal arabinose must have been located in position 3 of the 1,6-linked galactan side chains. The decrease in the 1,5-linked arabinose and the increase of terminal galactose after degradation with *endo*-arabanase indicated that the arabinogalactan must carry short, 1,5-linked araban chains, possibly at the end of the 1,6-linked galactan chains. There was no 1,4-linked galactose. An unknown peak, delineated by the question mark in Table 2 and eluting from DB 225 between terminal galactose and 2,4-branched rhamnose, was probably due to incomplete methylation.

Treatment with *exo*- and *endo*-arabanase followed by partial acid hydrolysis led to different lower molecular weights, which are given in Fig. 4. The shifts to lower molecular weights are difficult to interpret. Although arabinosidase and *endo*-arabanase both reduced the arabinose content of the arabinogalactan to nearly the same level (5·0% and 7·8% respectively), their molecular weight distributions are quite different. Treatment with arabinosidase led to a clear shift to *c.* 30 000 Daltons and to a shoulder at 50 000 Daltons, whereas the *endo*-arabanase-treated polymer shows one maximum at 40 000 Daltons.

Treatment with *exo*- and *endo*-arabanase could not remove arabinose completely (see Table 1 for alditol acetates): this was achieved by partial acid hydrolysis. The hot, diluted TFA further shifted the maximum of the arabinosidase-treated 1,3-1,6-linked galactan to 20 000 Daltons. The pure backbone consisted of about 120 galactose units, and it was difficult to hydrolyze. As Table 1 shows, the yield of neutral sugars did not exceed 75%, which often appears during the hydrolysis of completely β-conformed polysaccharides. The main linkage types are the 1,3-1,6-branching unit, and the

**Table 1. Monosaccharide composition of native and PE/PG-degraded juice pectin and the different DEAE-fractions. Values are given in % by weight**

| | MF/UF native pectin | PE/PG | DEAE | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | $-H_2O$ | −50 mM | −50 mM Arabinosidase | −50 mM *endo*-Ara | −50 mM Arabinosidase partial acid hydrolysis | −500 mM | −500 mM Arabinosidase | −500 mM *endo*-Ara |
| Rha | 0·9 | 2·9 | 6·6 | tr | tr | tr | — | 6·7 | 11·2 | 10·1 |
| Fuc | — | — | tr | — | — | — | — | — | — | — |
| Ara | 11·8 | 21·2 | 27·3 | 24·7 | 5·0 | 7·8 | — | 27·1 | 14·5 | 12·9 |
| Xyl | — | tr | 1·1 | — | — | — | — | tr | 1·3 | 1·1 |
| Gal | 17·9 | 55·3 | 35·6 | 67·1 | 85·8 | 89·1 | 75·0 | 24·0 | 36·0 | 36·1 |
| Glu | 2·9 | 1·7 | 11·7 | tr | tr | tr | — | tr | 1·5 | 1·9 |
| Total sugar | 33·5 | 81·1 | 82·3 | 91·8 | 90·8 | 96·9 | 75·0 | 57·8 | 65·6 | 62·1 |
| GalA[a] | 49·0 | 15·0 | 2·0 | 3·0 | — | — | — | 11·2 | 13·2 | 10·9 |
| Ratio Ara/Gal | 0·66 | 0·38 | 0·77 | 0·37 | 0·06 | 0·09 | — | 1·13 | 0·40 | 0·36 |

tr = Trace.
[a]Determined with *m*-phenylphenol.

**Table 2. Methylation analysis of the isolated polymers**

| Methyl-ether | DEAE $-H_2O$ | DEAE | | | | DEAE | | |
|---|---|---|---|---|---|---|---|---|
| | | −50 mM | −50 mM Arabinosidase | −50 mM *endo*-Ara | −50 mM Arabinosidase partial acid hydrolysis | −500 mM | −500 mM Arabinosidase | −500 mM *endo*-Ara |
| 234 Rha | — | — | — | — | — | 6·5 | 7·5 | 7·1 |
| 34 Rha | — | — | — | — | — | 2·4 | 9·4 | 7·9 |
| 24 Rha | — | — | — | — | — | 1·1 | 1·6 | 1·5 |
| 3 Rha | 1·3 | — | — | — | — | 4·1 | 2·4 | 2·2 |
| 235 Ara | 23·4 | 19·0 | 1·1 | 7·2 | — | 25·1 | 7·0 | 6·0 |
| 25 Ara | — | — | — | — | — | 4·5 | 5·3 | 5·0 |
| 23 Ara | 11·2 | 7·7 | 1·2 | — | — | 16·7 | 6·3 | 2·8 |
| 2346 Gal | — | 1·8 | 3·3 | 8·2 | 12·3 | 1·7 | 5·7 | 4·5 |
| 246 Gal | 13·4 | 16·0 | 18·8 | 12·6 | 24·1 | 8·1 | 6·8 | 7·5 |
| 234 Gal | 8·5 | 13·0 | 24·4 | 38·2 | 28·6 | 4·0 | 15·0 | 17·1 |
| 26 Gal | 1·6 | 1·3 | 2·0 | 2·2 | 2·9 | 2·2 | 2·7 | 3·3 |
| 24 Gal | 33·8 | 37·4 | 16·9 | 28·0 | 25·1 | 22·4 | 21·3 | 27·5 |
| 236 Glu | 5·0 | — | — | — | — | — | 1·8 | 1·5 |
| ? | 1·3 | 3·9 | 16·9 | 1·0 | 7·0 | 1·1 | 7·3 | 6·1 |
| 234 Xyl | — | — | — | — | — | — | — | — |

linear 1,3- and 1,6-linked chains. In Table 2, the corresponding methyl ethers of the main linkage types β-1,3-, β-1,6-gal and the branching unit β-1,3-β-1,6 gal are arranged in equal amounts.

The suggested structure was confirmed by $^{13}$C-NMR-spectroscopy: the spectra showed distinct differences to a type I arabinogalactan isolated from lupin seed (Carre *et al.*, 1985; Döring, 1989; Will, 1990). The backbone of type I-arabinogalactanes consisted of β-1,4-linked galactose. Arabinogalactans and arabinogalactan-proteins are widely distributed plant polysaccharides or proteoglycans. The proposed structures are similar to those from Keegstra *et al.* (1973), who found type II arabinogalactans in suspensions of cultured sycamore cells. Brillouet *et al.* (1990) isolated a similar polysaccharide from red wine. Gleeson *et al.* (1989) found them in Italian ryegrass and Cartier *et al.* (1987) isolated the polymer from suspension-cultured blackberry cells. The arabinogalactans are part of the hairy regions. In contrast to the rhamnogalacturonan backbone, the smooth regions of the pectin consist mainly of galacturonic acid. DeVries *et al.* (1982) established this current pectin model, where the side chains are arranged blockwise within the linear backbone of the galacturonic acid main chain.

The sugar composition and methylation data confirm fraction DEAE 500 mM as the rhamnogalacturonan zones of the pectin molecule, where the arabans are associated with the pectin backbone. High amounts of 1,5-linked arabinose in the presence of different rhamnose methyl ethers are in agreement with this assumption. Galacturonic acid was not transformed to

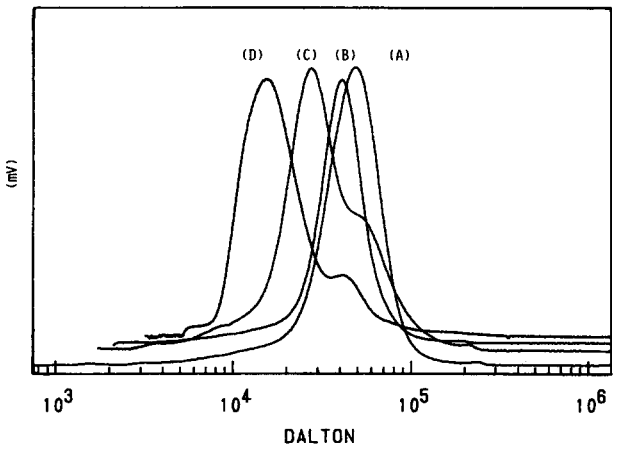


**Fig. 4.** Molecular weight shifts of the arabinogalactan: (A) untreated, (B) after *endo*-arabanase, (C) after arabinosidase, (D) after arabinosidase and partial acid hydrolysis.

1,4-linked galactose by carboxyl reduction and could therefore not be detected by methylation analysis.

The decrease in 3-Me-Rha and increase in 34-$Me_2$-Rha after degradation with both arabanases indicated the 1,4-linked galacturonic acid sequence was interrupted by 1,2-linked rhamnose, which carries at position 4 the branching point for linear 1,5-arabans. The arabinose content of this fraction could not be reduced in the same manner as for the arabinogalactan, possibly because of steric hindrance preventing the action of the enzymes. The high moieties of terminal arabinose and different galactose methyl ethers are strongly suggestive of arabinogalactans: therefore it seems that they appear more frequently in the rhamnogalacturonan zones. Voragen and Pilnik (1989) proposed a more detailed structure, where 1,4-linked galacturonic acid and 1,2-linked rhamnose are the main constituents of the rhamnogalacturonan backbone. They observed no unsubstituted galacturonic acid: the degree of esterification was 40 and the degree of acetylation 60%.

Fraction $-H_2O$, which accounted for 8% of the total DEAE-separated colloidal substances was similar to the arabinogalactan for fraction −50 mM, except for the peak in the molecular weight distribution below 10 000 Daltons. This peak ($-H_2O$ LM, Fig. 4) was collected separately and subjected to methylation analysis. The results (see Table 2) showed an enrichment of xyloglucan beside the already known arabinogalactan. In the common cell wall models (Albersheim, 1975; Wilson & Fry, 1986; Renard *et al.*, 1990), xyloglucans with a core of 1,4-linked glucose form the connections between the cellulose microfibrils and the neutral side chains of the middle lamella pectin. When high mechanical stress acts on the apple mash, as occurs during belt pressing on subsequent extraction with water, xyloglucans pass into the juice (Schopplein, 1989). It is still not fully clear what is the nature of any links between xyloglucan, pectin and cellulose of the plant cell wall (Renard *et al.*, 1990).

During storage of solutions of fraction DEAE $H_2O$ in water at 4°C, a sediment appeared after 3 days. After centrifugation and freeze drying the sediment was identified as a linear α-L-1,5-araban with a molecular weight of about 20 000 Daltons. In methylation analysis, the prominent methyl ether was 2,3-$Me_2$-Ara (86·5% of total, Will, 1990). Linear α-1,5-arabans are responsible for haze formation in apple juice concentrate (Schmitt, 1985).

## Practical consequences

The principal technological influences of carbohydrate juice colloids are a reduction in clarity and filterability, haze formation during storage and precipitation which can be predicted by accelerated testing using ethanol or 2-propanol. In addition to the composition and structure of the polymers their possible influences on juice technology had to be studied.

## Filtration tests

Filtration tests were performed on a laboratory scale. Solutions of the native juice pectin (MF/UF), the arabinogalactan (DEAE 50 mM) and the rhamnogalacturonan (DEAE 500 mM) were prepared at concentrations of 0·02%, which is comparable to the average colloid content of a clear apple juice. Only the native juice pectin showed a high viscosity (2·6 cP): the values of the arabinogalactan and the rhamnogalacturonan were 1·06 and 1·08 cP respectively (Ubbelohde capillary viscometer).

The clear solutions were membrane filtered (Seitz, pore size 0·45 μm, diameter 47 mm), and the filtration volume was plotted versus time. As shown in Fig. 5, in the case of the arabinogalactan there was no inhibitory effect on filtration. Nearly the same filtration rate was observed for the native juice pectin, which had the

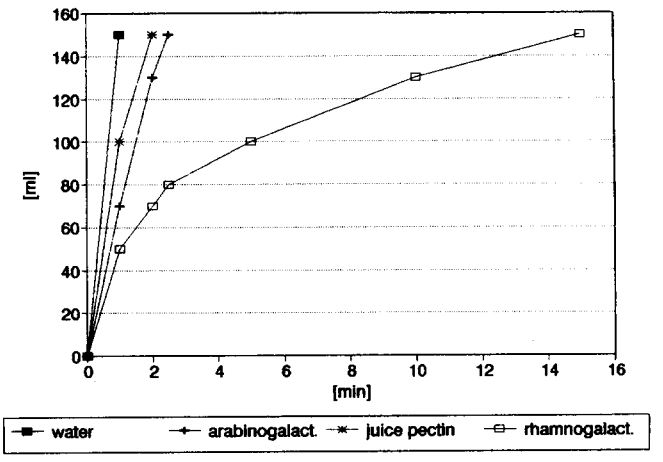


**Fig. 5.** Relative viscosities and filtration plots of the 0·02% colloid solutions.

highest viscosity at this concentration. In spite of its low viscosity the rhamnogalacturonan juice filtered much more slowly than the native pectin. It should therefore be underlined that treatment of juice pectin with purified PE/PG released pectic fragments, which caused filtration problems.

**Precipitation tests**

In the fruit juice industry stability tests normally depend on the simple ethanol test. For that purpose one part juice is mixed with one part ethanol. After the ethanol addition there should not be any precipitation within a definite time. A slight haze formation is normally acceptable. To confirm the behavior of the isolated polysaccharides under these conditions, 0·1% solutions acidified to pH 3·5 with citric acid were prepared and mixed with increasing parts of absolute ethanol. The arabinogalactan precipitated immediately at an ethanol concentration of 50% by volume: using 40% ethanol concentration, it took 30 min for precipitation to occur. The same could be observed with PE/PG sample, which remained after treatment of the juice pectin with the purified pectinases (without DEAE-fractionation). In the case of the rhamnogalacturonan direct precipitation was observed at a 60% ethanol concentration: at 50% the polysaccharide precipitated after 30 min.

**Degradation with technical enzyme preparations**

Treatment of the native juice pectin with purified PE/PG released the polymers described. In further studies their possible degradation with desalted technical pectinases was investigated.

**Arabinogalactan**

Two well known products (Rohapect 5DL, Röhm, Germany and Pectinex Ultra SP, Novo Nordisk, Switzerland) were used. In both cases the arabinose content of the polymer was reduced from 24·7 to around 6%. The same happened after treatment with purified arabinosidase. The $\beta$-1,3-$\beta$-1,6-galactosyl backbone remained unchanged. In parallel trials a $\beta$-1,4-galactan, isolated from lupin seed (Döring, 1989), was degraded completely. Normally technical enzyme preparations contain *endo*-arabanase- as well as arabinosidase-activity for the cleavage of linear arabans, which are responsible for haze formation in apple juice or concentrate. There was no activity on $\beta$-1,3- or $\beta$-1,6-galactan chains.

**Rhamnogalacturonan**

Owing to the filtration problems already described, the capacity of technical enzyme preparations to degrade rhamnogalacturonan seemed to be more important than the capacity to degrade arabinogalactan. Treatment with both enzyme preparations was monitored by the molecular weight distribution on Superose 12. The high molecular weight zones (>50 000 Daltons, see Fig. 3) of the polymer, which were responsible for the filtration problems, remained nearly unchanged after the action of Rohapect 5 DL. On the other hand Pectinex Ultra SP achieved a nearly complete degradation of the zones between 100 000 and 300 000 Daltons. The same results were obtained by Voragen (1990), who found only one out of 40 technical enzyme preparations which was able to depolymerize this substrate. The activity responsible for the cleavage of the rhamnogalacturonan was isolated, purified and determined as 'rhamnogalacturonase' (RGase) by the authors. Meanwhile more commercial and test products containing RGase-activity were found (Schols *et al.*, 1990*a*; F. Will, unpublished results).

## DISCUSSION

The results confirm the pectin model established earlier by groups of Albersheim and Pilnik. The blockwise arrangement of smooth and hairy regions, associated with arabans and arabinogalactans as postulated from DeVries *et al.* (1982) for the pectin from the AIR of apple mash can also be applied to the pectic polysaccharides from apple juice. *Endo*-PG together with PE only cleaves the smooth regions within the pectic molecule, consisting of partly methylated polygalacturonic acid. By combined PE/PG-action the arabinogalactans are released, and the rhamnogalacturonan zones remain untouched.

In most of the common technical enzyme preparations, PE, PG and PL (pectinlyase) are the main activities. The side activities of the preparations are not able to depolymerize the pectic polysaccharides completely during juice clarification (Dietrich, 1986). The filtration and precipitation tests illustrated the problems caused by the presence of the remaining pectin fragments with respect to clarification and stabilization of the final product. Meanwhile most of the technical pectinases contain *endo*- and *exo*-arabanase-activities, and therefore the araban-caused haze formation in apple juice concentrates disappeared. This problem often appeared during storage of apple and pear juice concentrates.

Clarification problems mainly appear during the manufactring of extracted apple juice. Extracted apple juices contain up to 10 times the amount of pectin compared with normal juices. In apple juice plants, water extraction is common practice for increasing the juice yields. Existing evaporation capacities make the concentration of the thin extraction juice economically justified. Belt pressing of the mash followed by water extraction of the cell wall material indeed leads to high

juice yields, but also to increasing colloid contents (Schols *et al.*, 1991). As a result of this, clarification and filtration problems previously described can occur, and the possible use of enzyme preparations with specific activities is required.

At the moment, only a few technical enzyme preparations containing for example rhamnogalacturonase (RGase) are available. Enzymic juice depectinization has to be finished after a maximum of one hour (hot clarification at 50°C). The degradation of the rhamnogalacturonan backbone with RGase requires enzymic deacetylation and/or dearabinosylation (Schols *et al.* (1990*b*). It seems doubtful that the synergistic action of acetylesterase, arabanases and RGase can carry out the degradation within the given time. The kinetics have to be studied under these conditions.

The type II arabinogalactan caused problems for the stability tests with ethanol. During fruit juice processing, ethanol tests are employed at two stages, firstly to control pectin degradation after pressing and enzyme treatment and, at the end of the processing line, to examine the colloidal stability of the finished juice rediluted from the concentrate. The main purpose is controlling pectin degradation. The degree of haze formation during the tests depends on the applied technology like pressing system, liquefaction, pump enzyming and the conditions of water extraction.

Stability tests are a general problem for the fruit juice industry, because no standardized specifications exist with respect to pH or to ethanol concentration. Companies apply a great variety of alcohol tests. Precipitation reactions of the carbohydrate polymers during stability tests of the finished juice also have negative economic effects in respect of the price fixing of the products. Methylation analysis of authentic samples from alcoholic precipitations of apple juices always indicated type II-arabinogalactans (F. Will, unpublished results), and therefore an enzymic degradation of the polymers during pectin degradation seems to be desirable.

The solution of the type II arabinogalactan did not affect the membrane filter test. This test works in the 'Dead-end-manner'. There is still less information about the behavior of the pectin fragments in modern filtration techniques like cross-flow micro- or ultrafiltration. Belleville (1991) found a great influence of pectin substances like arabinogalactans and rhamnogalacturonans on the formation of the membrane layer during cross-flow microfiltration of red wine. Investigations about the influence of arabinogalactans and rhamnogalacturonans from apple on cross-flow filtration are going on.

*Endo*-$\beta$-1,4-galactanase, purified from Pectinex Ultra SP (Schopplein, 1989), could not liberate galactose from the isolated polysaccharides. In contrast to the results of Knee *et al.* (1975) and DeVries *et al.* (1983), 1,4-linked galactose was not detected during methylation analysis. The only explanation for this fact is the different original material. Both groups used the AIR of the fruit tissue. In the present work the polysaccharides of the extracted juice were investigated. A dependence on apple variety seems to be improbable. However, until now there are no technical enzyme preparations with activities to the $\beta$-1, 3-$\beta$-1,6-galactan-core of type II-arabinogalactans.

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