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Analytical comparison of three industrial pectin preparations

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The chemical composition of three industrial 'rapid-set' pectin samples, one from apple and two from lemon, has been extensively investigated. The so-called 'ballast' that has been removed by copper precipitation appears to be mainly constituted of neutral polysaccharides and, to a less extent, of proteins and phenolic compounds. Purified pectin molecules are composed of galacturonic acid and neutral-sugar residues and also carry some phenolic and proteinaceous material. Pectin molecules of industrial apple and citrus preparations are very similar to those extracted under mild conditions from similar sources but carry fewer neutral sugars. All three pectins have been found to be slightly acetylated (DAc 1·5 and 5·0% for lemon and apple pectins, respectively). The pectin extracted from apple contains more neutral sugars and more phenolics but fewer proteins than the two lemon pectins, which are very close to each other. However, one of the lemon pectins contains a pectin fraction that appears to be less esterified. The same lemon pectin also differs from the other pectin samples by its higher calcium-ion content. Both structural and analytical consequences are discussed.

INTRODUCTION

Pectic substances are glycosidic macromolecules that occur exclusively in plants. They are industrially extracted to be used as food additive (Nelson *et al.*, 1977; Thom *et al.*, 1982; May, 1990). One of the greatest difficulties in their optimal utilization is that some industrial pectin preparations that appear to be very similar on gross analysis exhibit different physical behaviour. A better knowledge of the chemical structure of industrial pectins is thus of major importance to understanding and improving their technological applications.

The chemical structure of pectic substances has been intensively studied for more than 60 years because of their great importance in many fields. They act as 'lubricating' or 'cementing' agents in the cell-walls of plants (Rees & Wight, 1969). Pectic substances are involved in the interactions between plant hosts and their pathogens (Talmadge *et al.*, 1973; Albersheim *et al.*, 1981). The amount and nature of pectin strongly influence the texture of fruits and vegetables on growing, ripening, and storage (Voragen & Pilnik, 1970; Knee, 1978*a*, 1978*b*) and also affect the processing of fruits and vegetables (Rombouts & Pilnik, 1978). Pectin is a nutritional fibre (Cummings *et al.*, 1979) and possesses many interesting medical properties (Deuel & Stutz, 1958; Verstraete, 1979). Curiously, relatively little work dealing with the chemical composition and structure of industrial pectins has been published.

Pectin is primarily a linear polymer of D-galacturonic acid units joined by α(1–4)glycosidic linkages. The pyranose ring of D-galacturonic acid occurs in the chair form $^4C_1$ corresponding to the most stable conformation (Deuel & Stutz, 1958; Rees & Wight, 1971; Morris *et al.*, 1975). The glycosidic bonds at C-1 and C-4 are therefore in the axial–axial position.

Only a few examples of the isolation of pure homogalacturonan fractions are reported (Bishop, 1955; Zitko & Bishop, 1965; Aspinall & Canas-Rodriguez, 1958; Bhattarcharjee & Timell, 1965; Chambat & Joseleau, 1980). However, these fractions were obtained by severe treatments probably generating artifacts (Aspinall, 1980; de Vries *et al.*, 1982). From many studies, it has become clear that pectin is not a homo-

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polysaccharide but that neutral sugars are part of the pectin molecules.

L-Rhamnosyl residues are present in the galacturonan backbone (Aspinall & Fanshave, 1961; Barrett & Northcote, 1965; Aspinall *et al.*, 1967*a*, 1967*b*, 1968*a*, 1968*b*, 1969, 1970; Stoddart *et al.*, 1967; Foglietti & Percheron, 1968; Talmadge *et al.*, 1973; Kato & Noguchi, 1976; Toman *et al.*, 1976; Simson & Timell, 1978; McNeil *et al.*, 1980; Eda & Kato, 1980; Lau *et al.*, 1985; Sun *et al.*, 1987; Schols *et al.*, 1990), inserted in the following manner: *O*-α-D-GalA-(1-2)-*O*-O-L-Rha-(1-4)-*O*-A-D-GalA. Recent NMR studies on apple-pectin fragments have proved that the configuration of the L-rhamnosyl linkage is α (Colquhoun *et al.*, 1990).

Other neutral sugars, mainly L-arabinose, D-galactose, D-xylose, and D-glucose occur as side chains (Aspinall & Canas-Rodriguez, 1958; Neukom *et al.*, 1960; McCready & Gee, 1960; Aspinall & Fanshave, 1961; Barrett & Northcote, 1965; Zitko & Bishop, 1965; Hatanaka & Ozawa, 1966; Jacin *et al.*, 1967; Aspinall *et al.*, 1968*b*; Foglietti & Percheron, 1968; de Vries *et al.*, 1982; Schols *et al.*, 1990). The neutral side chains are linked to the main chain via the C-4 of the rhamnose units (Aspinall *et al.*, 1967*a*; Talmadge *et al.*, 1973; Eda & Kato, 1980; McNeil *et al.*, 1980; Lau *et al.*, 1985; Sun *et al.*, 1987; Schols *et al.*, 1990). However, galactose, arabinose, or xylose have often been found covalently linked to galacturonic acid (Bouveng, 1965; Barrett & Northcote, 1965; Aspinall *et al.*, 1967*a*, 1967*b*, 1968*a*; Stoddart *et al.*, 1967; Foglietti & Percheron, 1968; Kikuchi & Sugimito, 1976; Ishii, 1981; Schols *et al.*, 1990).

Some of the carboxyl groups of the rhamnogalacturonan backbone are esterified with methanol and some hydroxyl groups at C-2 and C-3 of the galacturonic units are esterified with acetic acid (McCready, 1970; Kim *et al.*, 1978; Rombouts & Thibault, 1986*a*). The free-acid groups may be partly or fully neutralized with sodium, potassium, calcium, or magnesium (Kawabata, 1977).

It has recently been shown that pectin molecules from spinach (Fry, 1983) and sugar beet (Rombouts & Thibault, 1986*a*) also carry some feruloyl groups. They are located mainly in the side chains (Rombouts & Thibault, 1986*b*) and may be covalently bound to the non-reducing termini of arabinose and/or galactose chains (Fry, 1983).

L-Fucose (Aspinall *et al.*, 1967*b*, 1968*a*, 1968*b*; Lau *et al.*, 1985), apiose (Darvill *et al.*, 1978), and some other rare sugars (Aspinall *et al.*, 1958, 1961; Barrett & Northcote, 1965; Foglietti & Percheron, 1968; Darvill *et al.*, 1978) were found as trace constituents in certain pectic substances, but there is no information available about their structural location.

In this work, the chemical composition of three industrial pectins was determined by using modern analytical methods. This includes minerals, acetyl esters, protein, and amino acids, which are often neglected in the characterization of pectin preparations.

EXPERIMENTAL

Material

Three unstandardized industrial pectins classified as 'rapid-set' were obtained from Sanofi Bio Industries (France): two from lemon peels (A and B) and one from apple pomace. All three were industrially extracted by the same classical hot-acid procedure (May, 1990).

Copper purification

Commercial samples (0·5% solutions, 1000 ml) were purified by precipitation with a 7% aqueous copper acetate solution (75 ml). The precipitate that formed was collected on a G3 fritted-glass filter and abundantly washed with distilled water. The precipitate was then redissolved in 20 mM Na-CyDTA (titriplex IV, Merck) at pH 5, dialysed against distilled water, and freeze-dried.

Analytical methods

The anhydrouronic acid content (M = 176) was determined by the automated *meta*-hydroxydiphenyl assay (*m*hdp, Thibault, 1979).

Underivatized uronic acid units were analysed by HPLC after hydrolysis of the pectin samples in 2N H_2SO_4 at 100°C for 3 hours. HPLC analysis was performed with a Dionex system (Sunnyvale, CA, USA) equipped with a CarboPac PA1 column (9 × 250 mm) and a pulsed amperometric detector. Conditions were very similar to those used by Martens & Frankenberger (1990).

Neutral sugars were determined as their alditol acetates by GLC, 2-mg samples being hydrolysed for 1 hour at 121°C with 2N TFA (Albersheim *et al.*, 1967). Hydrolysates were dried under a stream of air at 40°C, reduced with $NaBH_4$ (10 mg) in 1·5N ammonia (0·2 ml), and acetylated with acetic anhydride (3 ml, 30 min, room temperature) in the presence of 1-methyl imidazole (0·45 ml) as catalyst (Blakeney *et al.*, 1983). The alditol acetate derivatives of the sugars were then separated from the aqueous phase by extraction with dichloromethan (2 × 3 ml) and determined by GLC equipped with an OV 275 packed column.

The methoxyl and the acetyl contents were determined by HPLC analysis of the methanol and the acetic acid released on alkaline de-esterification (1 hour, 5°C, 0·5M KOH; Voragen *et al.*, 1986). The degree of methoxylation (DM) and the degree of acetylation (DAc) were calculated by using the AGA content measured by the *m*hdp assay.

The total nitrogen content was determined by a semi-automated micro-Kjeldhal method. Protein content was estimated by multiplying the N content by 6·25.

Amino-acid compositions were determined with a Biotronic LC 600 E automatic analyser. Samples were hydrolysed in 6M HCl for 21 hours at 110°C under nitrogen.

Starch content was determined enzymatically by using a test kit (Boehringer, Mannheim, FRG).

Total phenols were determined with the Folin–Ciocalteu reagent without copper treatment and with ferulic acid used as standard (Swain & Hillis, 1959), 0·25M Folin–Ciocalteu reagent (0·4 ml) being added to the sample solution (0·5%, 0·4 ml), followed after 5 min by 1M Na_2CO_3 (0·4 ml). The absorbance at 750 nm was read after 1 hour.

Phenolic acids were assayed by HPLC. Pectin samples (100 mg) were treated with 0·5M KOH (5 ml) in a screw-cap tube under N_2 at room temperature for 24 hours with *p*-hydroxybenzoic acid as internal standard, after which 6M HCl (0·75 ml) was added to the mixture, and the phenolic components were recovered from the acidified solution by extraction with ethyl acetate (2 × 4 ml). The combined ethyl acetate extracts were dried under vacuum at 40°C, the residues were dissolved in methanol (1 ml), and aliquots (20 µl) were injected on a reverse-phase Spherisorb 10 ODS column (Chrompack, 250 × 4·6 mm). The column was eluted with a linear-gradient mixture of 4% (v/v) AcOH in MeOH/4% (v/v) AcOH in H_2O (10–50%) at a flow rate of 1·5 ml/min. The eluate was monitored in UV at 280 nm.

Sodium, potassium, and calcium were simultaneously determined with an ELEX 6361 flame AES (Eppendorf), magnesium with an IL 357 flame AAS (Instrumental Laboratory), and phosphorus colorimetrically with molybdene blue (ISO 3946-1982, UDC 664-2:543·847).

Molecular-size distributions were determined by high-performance size-exclusion chromatography (HPSEC), a series of Biogel TSK columns 60XL, 40XL, and 30XL (300 × 7·5 mm) being used in combination with a Biogel TSK guard column (75 × 7·5 mm). Columns were eluted with 0·M Na-acetate buffer at pH 3·0 with a flow rate of 0·8 ml/min at 30°C and detected with a Shodex SE 61 RI detector at 40°C.

Charge distributions were determined by high-performance ion-exchange chromatography (HPIEC) as described by Schols *et al.* (1989). A Biorad MA7P column (50 × 7·8 mm) was eluted with a linear gradient of 15–270mM Na-phosphate buffer at pH 6·0 at a flow rate of 1·5 ml/min. Detection was done by reading the absorbance in UV at 215 nm. The increase in baseline signal was corrected by subtracting the chromatogram obtained for a blank run from those of sample runs.

RESULTS AND DISCUSSION

Three unstandardized industriial pectin samples, one from apple pomace and two from lemon peels, empirically known for their different solubility and gel behaviour, especially in the presence of calcium, have been extensively investigated for their chemical composition.

Uronide residues

All three industrial samples contain a high amount of uronide (Table 1) as evidenced by the *m*hdp assay. Moreover, HPLC analysis of pectin hydrolysates did not reveal the presence of any uronic acid other than galacturonic acid. This does not confirm the identification of some glucuronic acid in pectins by Aspinall *et al.* (1967*b*, 1968*a*, 1968*b*) and McNeil *et al.* (1980). Since glucuronic acid units are probably accommodated in side chains (McNeil *et al.*, 1980), they may have been removed from the native pectin during the industrial-extraction process. However, complete

Table 1. Composition (as percentage weight of dry matter) of the unstandardized industrial pectin samples

	Lemon A	Lemon B	Apple
Galacturonic acid[a]	76·4	77·1	60·8
Methoxyl groups[b]	4·4(71·5)	4·4(72·1)	3·6(74·3)
Acetyl groups[b]	0·26(1·4)	0·30(1·6)	0·72(5·0)
Total neutral sugars[a]	8·5	9·2	27·0
Proteins (N × 6·25)	3·0	3·3	1·6
Total phenols	0·18	0·15	0·59
Ash	2·38	1·96	1·89
Total	95·1	96·3	95·9

[a]Values recorded as 'anhydro' residues.
[b]Values in parentheses are degree of methoxylation (DM) or degree of acetylation (DAc).

hydrolysis of the galacturonan backbone was not achieved since some oligomeric fragments were detectable. It is thus possible that glucuronic acid residues remained attached to oligomers that could not be resolved by the HPLC analysis.

The apple-pectin sample contains less galacturonic acid (60·8%) than the two lemon pectins (76·4 and 77·1% for lemon A and lemon B, respectively). This is mainly due to the difference in neutral-sugar contents (Table 1).

Neutral sugars

The industrial apple-pectin preparation contains about 25 times as many glucose, 12 times as many xylose, four times as many arabinose, and 1·5 times as many rhamnose residues per 100 galacturonide residues as the lemon-pectin samples (Table 2). However, purification with copper ions shows that these neutral sugars are not all covalently bound to the pectin molecules.

Copper precipitation removed more than 60% of the glucose units present in the industrial apple pectin. Most of it is of starch origin, which represents 10·5£ (dry weight) of the industrial apple pectin. This justifies the enzymatic removal of starch molecules, performed in some factories in order to avoid too large a dilution of industrial apple pectins and potential problems of precipitation on application (May, 1990). Commercial lemon pectins also contain some starch (0·16 and 0·51% for lemon A and lemon B pectins, respectively) but in much lower quantity than the apple pectin. In the apple pectin, starch accounts for more glucose than that which has been removed by copper precipitation (7·6% dry weight), indicating that, despite intensive washing, some starch could not be removed from the copper pectinate precipitate.

Copper precipitation also removed almost all mannose and parts of arabinose, galactose, and xylose units. The proportion of arabinose removed is higher than that of galactose, especially in the apple pectin. The removal of neutral-sugar residues by copper purification is due to the occurrence of free neutral polysaccharides, such as arabans, arabino-galactans, xyloglucans, or mannans, that have been co-extracted with the pectin fraction (Aspinall, 1980).

Some rhamnose units did not precipitate with copper ions from the three industrial pectins. The literature on plant cell-walls does not relate the occurrence of rhamnose units with any other polysaccharide than pectin. Moreover, some galacturonic acid did not precipitate either during the purification treatment (0·40, 0·34, and 1·45% from lemon A, lemon B, and apple pectins, respectively). This would mean that copper ions do not precipitate some rhamnose-rich pectin molecules, molecules that are known to carry many other neutral-sugar residues (McNeil *et al.*, 1980; de Vries *et al.*, 1981; Schols *et al.*, 1990). McCready & Gee (1960) and Michel *et al.* (1981) observed a similar loss of rhamnose and galacturonic acid during copper purification but with a much lower rhamnose/galacturonic acid ratio.

After purification, the sugar composition of the lemon B pectin is very close to that of the lemon A pectin, both qualitatively and quantitatively, except for a slightly higher content of galactose. On the other hand, the apple pectin differs from the lemon pectins by its higher content of neutral sugars, especially glucose, xylose, arabinose, and rhamnose. This confirms that the neutral-sugar content appears to be determined by the plant source (Nelson, 1977; de Vries *et al.*, 1984*b*). These differences in neutral-sugar composition may explain some differences in molecular conformation and thus physical properties: rhamnose is thought to disturb the regularity of the galacturonan backbone (Rees & Wight, 1971; Talmadge *et al.*, 1973) and may play an important role in the formation of junction zones (Thom *et al.*, 1982); other neutral sugars constitute side chains, which may limit inter-chain associations. It is interesting to note that all three pectins still contain some mannose and xylose residues and significant amounts of glucose. Some starch molecules are still present in the purified apple pectin (see above). However, in all three pectins, the starch content of the industrial samples does not account for all the glucose present, indicating that some non-starchy

Table 2. Neutral-sugar composition (as mol/100 mol gal A) of the unstandardized industrial and corresponding copper-purified pectin samples

	Commercial samples			Cu-purified samples		
	Lemon A	Lemon B	Apple	Lemon A	Lemon B	Apple
Rhamnose	2·1	1·6	2·9	1·5	1·3	2·1
Arabinose	3·3	3·1	12·8	2·1	2·3	3·5
Xylose	0·2	0·2	2·1	0·1	0·1	1·9
Mannose	0·2	0·2	0·2	t	t	t
Galactose	6·8	7·7	8·9	3·8	4·8	3·8
Glucose	0·6	1·0	23·3	0·3	0·5	8·5
Total	13·2	13·8	60·2	7·8	9·0	19·8

glucose units may be bound to pectin molecules. Xylose and glucose are generally found in apple pectins (Zitko & Bishop, 1965; Knee, 1978*a*; de Vries *et al.*, 1981; Aspinall & Fanous, 1984). In lemon pectins, xylose is also present but in much lower quantity than in apple pectins (Aspinall *et al.*, 1968*a*; de Vries *et al.*, 1984*b*; Thibault *et al.*, 1988; Axelos *et al.*, 1989). Simultaneous occurrence of xylose and glucose in purified pectins has been ascribed to the presence of some xyloglucan fragments attached to the pectin side chains (Talmadge *et al.*, 1973; de Vries *et al.*, 1981). However, several observations (Aspinall *et al.*, 1968*a*; de Vries *et al.*, 1982; Schols *et al.*, 1990) indicate that some xylogalacturonan regions exist. Contrary to de Vries *et al.* (1981) but in agreement with Knee (1978*a*), Stevens & Selvendran (1984*a*), and Thibault *et al.* (1988), we have been able to detect some traces of mannose in all three purified pectin samples analysed. According to Leigh & Krzeminski (1966), mannose may arise from epimerization of glucose during the hydrolysis step of the neutral-sugar analysis. However, in the conditions we used, no mannose appeared during the analysis of a glucose control.

Compared with pectins of similar sources extracted in mild conditions (Knee, 1978*a*; de Vries *et al.*, 1981, 1984*b*; Stevens & Selvendran, 1984*a*; Aspinall & Fanous, 1984; Thibault *et al.*, 1988), these industrial samples contain relatively few neutral sugars. This seems to be a general feature of industrial pectins (Kawabata, 1977; Michel *et al.*, 1981; Axelos *et al.*, 1989), and it may be explained by the rather severe conditions applied to the pectin during its industrial extraction. Indeed, in hot-acid conditions, acid-labile bonds, especially arabinosidic linkages, undergo degradation, resulting in a 'trimming' phenomenon of the pectin side chains.

Methyl/acetyl esters

Table 1 shows the methoxyl and acetyl contents of the three industrial pectins. In order to maintain the mass balance, they were calculated by using masses of 14 and 43 g for methoxyl and acetyl, respectively. In fact, the polygalacturonic acid content was calculated by using a mass of 176 g (see above), irrespective of its degree of methylation and/or acetylation.

All three industrial pectins are highly methoxylated. The observed degrees of methoxylation (DM) are in agreement with those generally observed for both 'rapid-set' industrial pectins (Kawabata, 1977; Axelos *et al.*, 1989) and pectins from similar sources extracted in mild conditions (Aspinall *et al.*, 1968*a*; Knee, 1978*a*; de Vries *et al.*, 1984*b*; Stevens & Selvendran, 1984*a*), indicating that the industrial procedure does not affect the methoxyl esters very much. Since the two lemon pectins are very close to each other, the apple pectin differs from them by reason of its higher DM. That confirms the generally accepted fact (May, 1990) that industrial apple pectins have a higher DM than industrial citrus pectins.

The three industrial pectins also contain some acetyl esters. The amounts measured are higher than those described in the literature, especially for the apple pectin (McComb & McCready, 1957). However, Thibault *et al.* (1988) and Voragen *et al.* (1986) analysed citrus pectins with DAc values of the order of two. Although DAc values lower than 12·5% do not hinder gelation (BeMiller, 1986), it is likely that acetylation influences gel properties. This has been neglected in rheological studies on apple and citrus pectins. Moreover, a relatively high acetyl content may explain the differences generally observed between DM obtained by titration and that obtained by methanol determination. Indeed, with the titration procedure, acetyl residues released on saponification add up to esterified galacturonic acid units. For instance, a 70% methoxylated and 4% acetylated pectin would appear to be 71·2% methyl-esterified.

Proteins/amino acids

The protein content of the three industrial pectins is given in Table 1 and the amino-acid composition in Table 3.

Lemon A and lemon B pectin samples contain very similar amounts of protein, and their amino-acid compositions are very close to each other. However, lemon B pectin exhibits a lower content of asparagine and hydroxyproline but a higher amount of glutamine and leucine. On the other hand, apple pectin contains much less protein than the lemon pectins, and its amino-acid composition is clearly different. Apple

Table 3. Amino-acid composition (as mole%) of the unstandardized industrial pectin samples

	Lemon A	Lemon B	Apple
Asparagine	13·2	10·5	16·9
Threonine	6·2	6·7	7·2
Serine	5·4	5·3	6·4
Glutamine	10·3	12·7	15·2
Glycine	9·8	9·5	9·9
Alanine	7·1	7·3	8·9
Valine	5·7	5·7	4·5
Cystine	t	t	t
Methionine	t	t	t
Isoleucine	3·6	3·7	3·1
Leucine	6·2	7·0	6·1
Tyrosine	3·1	3·4	1·9
Phenylalanine	3·4	4·0	2·4
Lysine	6·7	6·6	4·1
Histidine	2·7	2·4	1·8
Arginine	3·9	3·8	3·4
Hydroxyproline	5·8	4·7	4·4
Proline	6·8	6·7	3·8

pectin contains less tyrosine, phenylalanine, lysine, histidine, and proline but more asparagine, glutamine, and alanine. Anderson *et al.* (1987) found very similar differences between industrial pectins from apple and lemon.

Copper precipitation removed about 35% of the proteinaceous compounds from lemon A pectin and 30% from lemon B pectin but none from apple pectin. Even after this purification, the protein contents of the lemon pectins remain significantly higher than that of the apple pectin.

The amino-acid composition of copper-purified pectins has not been investigated. It is thus impossible to draw any qualitative conclusion about the nature of the proteinaceous compounds that are attached to the pectin molecules. Moreover, although covalent bonds have already been identified between amino acids and neutral-sugar residues (Lamport, 1969; Lamport *et al.*, 1973), the mode of attachment of these proteins to the pectin molecules remains unknown.

The high content of hydroxyproline, proline, serine, threonine, valine, tyrosine, and lysine suggests that the proteinaceous compounds present in the industrial samples may be fragments of one of the various structural proteins that occur in plant cell-walls (Cassab & Varner, 1988).

The presence of asparagine and glutamine may explain the detection of some naturally occurring amidated uronide units in sunflower pectins (Lin *et al.*, 1976). Indeed, the ammonia released on alkaline treatment (National Research Council, 1972) may come from amidated amino acids. However, even the industrial lemon pectin A, which contains about 0·9 mg of asparagine and 0·7 mg of glutamine (if complete amidation is assumed), would appear to be less than 0·25%-amidated with the procedure of the National Research Council (1972). Moreover, since the association of proteins with pectic substances appears to be a general phenomenon (McNeil *et al.*, 1982; Stevens & Selvendran, 1984*a*), the Kjeldhal procedure does not fit with the accurate determination of the degree of amidation of industrial pectins. For instance, the lemon A industrial pectin, which contains 0·48% (w/w) of total nitrogen, would appear to be almost 8%-amidated with the Kjeldhal procedure.

Phenolics

The total phenol contents of the industrial pectin samples are given in Table 1. The apple pectin contains more than three times as many phenolic compounds as the lemon pectins. This may explain the brownish colour of apple-pectin solutions.

The composition of these phenolics has not been investigated. However, HPLC analysis did not reveal the presence of any phenolic acids, such as ferulic acid (Rombouts & Thibault, 1986*a*) or *p*-coumaric acid (Guillon & Thibault, 1988), which are known to occur in sugar-beet pectin.

Only part of the total phenolics could be removed by copper purification (39, 13, and 27%, for lemon A, lemon B, and apple pectins, respectively), which suggested that at least some phenolic compounds other than phenolic acids might be bound to the pectin molecules. Moreover, treatment with polyclar AT, which is often used to remove polyphenolic residues, did not remove any phenolic compounds from the industrial pectin samples. Since polyclar treatment also did not remove any glycoside, it seems that polyphenolic material present in industrial preparations is entirely complexed with pectic substances as suggested by Stevens & Selvendran (1984*b*) or with neutral polysaccharides.

Minerals

Table 4 shows the mineral composition of the three industrial pectin samples. The apple pectin differs from the lemon samples by reason of its higher content of potassium, magnesium, and phosphorus, and a lower content of sodium. The difference in magnesium content between apple and lemon pectins is not as marked as that observed by Kawabata (1977). The existence of phosphorous in these pectins has not been established, but Henglein *et al.* (1949) verified the occurrence of phosphoric acid associated with pectin via ester or ionic linkages. Whereas almost all phosphorus could be removed by percolation through a column of Amberlite IR 45 anion exchanger, the hypothesis of ionic linkage seems to be more reliable.

Among the three industrial pectin samples, irrespective of the plant source, the lemon A pectin is characterized by a much higher calcium content. That seems to indicate the presence of some regions that strongly retain calcium ions along with the pectin molecules of the lemon A sample, e.g. blocks of de-esterified galacturonide units that may have been created by the attack of native pectin esterase (Kohn *et al.*, 1968).

It appears that non-esterified galacturonic acids are only neutralized up to 44·4, 27·9, and 43·8% for the lemon A, lemon B, and apple pectins, respectively, with the cations that have been determined (see Table 4). Since all three industrial samples have been extracted

Table 4. Mineral composition (as percentage weight of dry matter) of the three industrial pectin samples

	Lemon A	Lemon B	Apple
Na^+	0·17	0·15	0·04
K^+	0·27	0·20	0·62
Ca^{2+}	0·74	0·36	0·31
Mg^{2+}	0·05	0·05	0·06
PO_4^{2-}	0·04	0·04	0·15

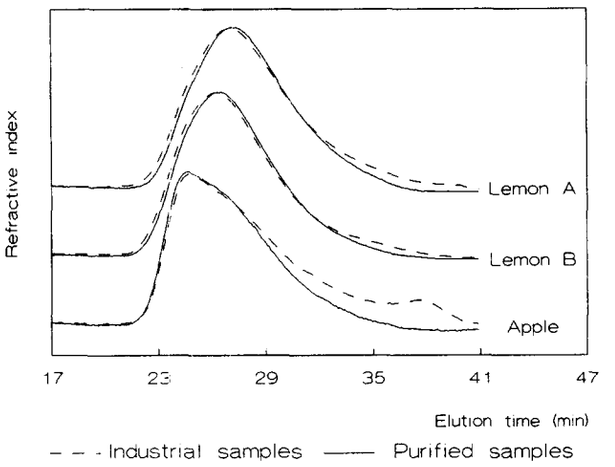

Fig. 1. HPSEC of industrial and purified pectin samples.

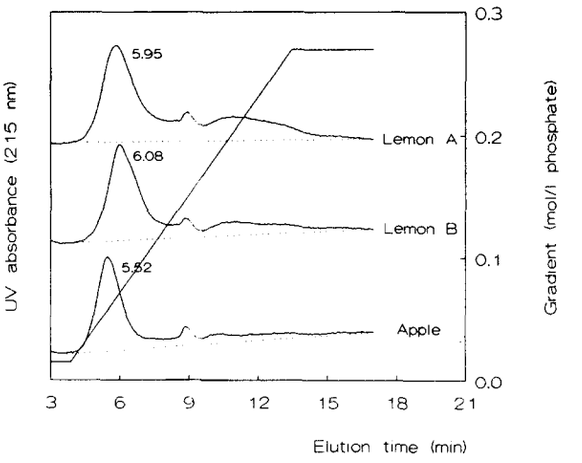

Fig. 2. HPIEC of industrial pectin samples on MA7P.

under the same conditions, no explanation can at present be given for this observation.

Molecular-size distribution

HPSEC was used for the rapid characterization of the pectin molecular size. Figure 1 shows the elution pattern of the three industrial pectin samples. The system has not been calibrated for molecular-mass determination on purpose. Indeed, SEC separates molecules according to their molecular size (Laurent & Killander, 1964). Calibration and subsequent determination of molecular mass are possible only for series of compounds of similar molecular shape and density. Chemical analysis has shown that apple pectin contains more neutral-sugar residues than lemon pectins. Side chains as well as rhamnose units, which increase the main-chain flexibility, may render apple-pectin molecules more compact and more dense than those of lemon and, at similar molecular mass, they should elute later because of their smaller molecular size. Without using specific detection, such as light scattering or on-line viscosity, it thus appears impossible to derive the molecular mass of pectins from HPSEC when the system is calibrated with pectins of different origin.

Lemon pectins were found to elute within one single peak. The apple pectin, however, exhibits a second peak of smaller size which elutes in the tail of the main peak. Differences in elution time indicate that the lemon B pectin sample has a larger hydrodynamic volume than the lemon A sample and that both lemon pectins have a smaller hydrodynamic volume than the apple pectin. This latter observation suggests a very high average molecular mass for the apple pectin, all the more as it is expected to have a relatively small molecular size compared to its molecular mass (see above). The broadness of the peaks also indicates that, within one pectin sample, the size of the molecules is not homogeneous.

Figure 1 also shows that copper purification mainly removed molecules that elute in the tail of the peak. This indicates that free neutral polysaccharides have a relatively low hydrodynamic volume. These results confirm those obtained by Michel *et al.* (1981) and Brigand *et al.* (1990).

Intermolecular charge distribution

The determination of degrees of methoxylation (DM) and acetylation (DAc) as described above provides only average values. Figure 2 shows the chromatograms obtained for the three industrial samples by high-performance ion-exchange chromatography on an MA7P column. As expected for highly methoxylated pectins (van Deventer-Schriemer & Pilnik, 1976; Schols *et al.*, 1989), the main bulk of the three samples elutes at a rather low ionic strength. However, some other peaks appear at a higher ionic strength. All three chromatograms exhibit a well-defined peak, eluting always at the same elution time (8·96 min). Schols *et al.* (1979) suggested that this peak is due to the elution of some pectin molecules under conditions that are not yet elucidated. We have isolated this peak by collecting the eluates from repeated runs and have found that it reacts positively with the *m*hdp test. However, since this peak also occurs during blank elutions, it could also be due to the elution of some salt impurities bound during the column regeneration at low ionic strength. Their elution at a given concentration of phosphate buffer may suddenly increase the ionic strength of the eluent and provoke the elution of some pectin molecules that should elute later. A third broader peak elutes after 9 min, i.e. high ionic strength. This peak is due to the elution of pectin molecules of low DM (Schols *et al.*, 1989).

The apple pectin that has the highest DM (Table 1)

elutes first. Surprisingly, the lemon B pectin, which is slightly but significantly more methoxylated than the lemon A, elutes somewhat later. This may be explained by the relative importance of the third peak in the lemon A pectin, indicating the presence of numerous low-esterified molecules that lower the average DM of the whole sample. Such molecules also occur in the lemon B pectin, but in a much lower proportion. They are almost absent in the apple pectin. The determination of the average DM of industrial pectins by ion-exchange chromatography (van Deventer-Schriemer & Pilnik, 1976; Schols *et al.*, 1989) is thus very inaccurate as soon as the distribution of DMs is not homogeneous. Low methoxylated pectin molecules exhibit a high affinity for calcium ions (Kohn, 1975), and their presence in larger proportion in the lemon A pectin sample may explain its higher calcium content as well as some gelling properties.

Copper purification did not change the elution pattern of the pectin samples on HPIEC, indicating that no significant de-esterification occurred during the treatment. This confirms the findings of Michel *et al.* (1981).

CONCLUSION

Apple and lemon industrial pectin preparations differ from each other in their chemical composition, both quantitatively and qualitatively. Apple pectin appears to contain more neutral sugars and more phenolics but fewer proteins than lemon pectins. HPSEC indicates that apple pectin also has a much larger molecular size than lemon pectins.

Copper purification proved that only part of these compounds is associated with pectin molecules. All three industrial pectin preparations contain free neutral polysaccharides (especially starch in the apple pectin), free phenolic compounds and free proteins which constitute the so-called 'ballast' (Michel *et al.*, 1981; Brigand *et al.*, 1990). The presence of such impurities results from the incomplete purification during the industrial extraction, probably because of the low specificity of the alcohol precipitation. Their effects on the physical properties of pectins remain unknown.

The presence of neutral sugars that could not be separated from the galacturonide fraction by copper purification indicates that industrial pectins may carry neutral side chains as has often been demonstrated for laboratory-extracted pectins. However, the degradative hot-acid conditions of the industrial extraction lead to a 'trimming' of the side chains. Side chains are thus shorter and/or less abundant in industrial pectins than in pectins from similar sources extracted under mild conditions.

It is interesting to note that purification tends to reduce compositional differences between apple and lemon pectins. Analytical differences between apple and lemon industrial pectins appear to be due more to the presence of accompanying molecules than intrinsic differences between the pectin molecules. However, the purified apple pectin clearly differs from the purified lemon pectins. Although differences may arise from the physiological state (Gould *et al.*, 1965; Knee, 1973, 1978*a*; de Vries *et al.*, 1984*a*) and extraction conditions (Joslyn & Deuel, 1963; de Vries *et al.*, 1981), our results seem to confirm that pectin composition depends very much on plant origin (Zitko & Bishop, 1965; Kawabata, 1977; de Vries *et al.*, 1984*b*). Apple-pectin molecules contain more neutral sugars and probably more phenolic compounds but fewer proteins than lemon pectin molecules.

Of the two lemon pectins studied, one has been found to retain many more calcium ions than the other.

Some of these analytical differences may explain some empirically known differences in physical behaviour that are of great technological importance. However, because of the many varying parameters, the establishment of the composition–properties relationship may require a statistical investigation carried out on a large number of industrial pectin samples. Moreover, the analysis of the whole molecules does not provide any information about differences between molecules (inter-molecular structure) or on the sequence of the different constituents (intra-molecular structure). These industrial samples have also been preparatively fractionated by gel-filtration and ion-exchange chromatography and have been specifically depolymerized by using both chemical and enzymic methods. These data will be the object of further papers in this series.

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