Carbohydrate Research, 154 (1986) 177–187
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

FERULOYLATED PECTIC SUBSTANCES FROM SUGAR-BEET PULP

FRANK M. ROMBOUTS* AND JEAN-FRANÇOIS THIBAULT

Institut National de la Recherche Agronomique, Laboratoire de Biochimie et Technologie des Glucides, Rue de la Géraudière, F-44072 Nantes (France)

(Received November 14th, 1985; accepted for publication, March 20th, 1986)

ABSTRACT

Pectins have been isolated from an ethanol-insoluble residue of sugar-beet pulp by sequential extraction with water, oxalate, hot dilute acid, and cold dilute alkali in yields of 2.2, 0.53, 20, and 11%, respectively. They were purified by chromatography on DEAE-cellulose at pH 4.8, or by precipitation with copper sulphate (alkali-soluble pectin). The pectins had fairly low molecular weights, a high degree of acetylation, and relatively high contents of neutral sugars, but there were clear differences between the four fractions. The main neutral sugars in each pectin were arabinose and galactose, and rhamnose, fucose, xylose, mannose, and glucose were also present. The fractions were homogeneous in ion-exchange and gel-filtration chromatography. Polyphenols (1–2%) and possibly proteins (3–6%) were associated with the purified pectins. In addition, feruloyl groups (up to 0.6%) were linked mainly to the acid-soluble and alkali-soluble pectins.

INTRODUCTION

Beet pulp, the residue left from ground sugar beet after extraction of the sugar, is normally pressed, dried, and used as cattle feed. With the increase in energy prices, more and more of the pressed sugar-beet pulp is put into silage, as was formerly done with the wet pulp. The material is rich in polysaccharides, particularly pectin, arabinan, galactan, and cellulose[1]. As its content of "anhydrogalacturonic acid" is ~25% of the dry weight, it is a potentially rich source of pectin. However, attempts in the past to commercialise sugar-beet pectins have failed, since they have poor gelling properties, compared to those from apple and citrus[2], ascribed mainly to the high content of acetyl groups and the relatively small molecular size[3].

Our recent studies of sugar-beet pectins have revealed some new features, which may increase their usefulness.

*Present address: Agricultural University, Department of Food Science, De Dreijen 12, 6703 BC Wageningen, The Netherlands.

0008-6215/86/$ 03.50

EXPERIMENTAL

Sugar-beet pulp. — Pressed sugar-beet pulp, stored under ethanol, was obtained from the "Général Sucrière" factory at Eppeville, France.

Preparation of alcohol-insoluble residue (AIR). — Drip-dry pulp (100 g) was finely ground with aqueous 95% ethanol (400 mL), the slurry was filtered, and the insoluble material was washed with aqueous 80% ethanol until the filtrate gave a negative reaction in the phenol–sulphuric acid test[4]. The residue was washed with aqueous 96% ethanol and then with acetone, and allowed to dry at room temperature to give AIR (12 g).

Extraction of pectic substances. — Each of the following four extractions was repeated three times. AIR (35 g) was stirred with distilled water (2.2 L) for 30 min at room temperature, the slurry was centrifuged at 3000*g*, and the supernatant solution (containing water-soluble pectin, WSP) was collected. The pellet was mixed for 30 min with aqueous 1% ammonium oxalate (2 L) and centrifuged, and the supernatant solution (containing oxalate-soluble pectin, OXP) was collected. The pellet was heated with 0.05M hydrochloric acid (2 L) for 30 min at 85°, then cooled, and centrifuged. The supernatant solution (containing acid-soluble pectin, HP) was collected and neutralised to pH 4.5 with 2M sodium hydroxide. Cold 0.05M sodium hydroxide (2 L) was stirred with the pellet for 30 min at 4°, the mixture was centrifuged, and the supernatant solution (containing alkali-soluble pectin, OHP) was neutralised to pH 4.5 with M hydrochloric acid.

Each pectin solution was concentrated at <40° to ~200 mL, treated with 4 vol. of aqueous 95% ethanol, and kept overnight at 4°. Each precipitate was collected on a G3 sintered-glass filter and washed with aqueous 95% ethanol, and a solution in distilled water was freeze-dried.

Purification of pectins. — Chromatography was performed on columns (26 × 3.2 cm) of Whatman DEAE cellulose DE 52 equilibrated at pH 4.8 with 0.05M sodium acetate buffer, by elution at 50 mL/h with a linear gradient (900 mL) of 0.05→0.8M sodium acetate buffer (pH 4.8; the molarities of acetate buffers refer to their ionic strengths). Only 200–300 mg of pectin could be loaded and, before reuse, the column was regenerated conventionally with acid and alkali.

To a solution of OHP (2 g) in distilled water (200 mL) was added aqueous 7% copper sulphate (15 mL). The precipitate was collected on a G2 sintered-glass filter and extensively washed with distilled water, and a solution in aqueous 2.5% ethylenediamine tetra-acetic acid disodium salt (Na_2EDTA, 200 mL) was dialysed twice against 4 L of 0.05M sodium acetate buffer (pH 4.8, 4 L) and twice against distilled water, and freeze-dried.

Analytical chromatography. — This was performed on columns (10.5 × 1.6 cm) of DEAE-Sepharose CL-6B (Pharmacia), equilibrated with 0.05M sodium acetate buffer (pH 4.8). After the loading of each sample of crude pectin (4 mL of a 2 mg/mL solution), the column was washed with buffer (50 mL) and then eluted

at 30 mL/h with a linear gradient (100 mL) of 0.05→0.8M sodium acetate buffer. Fractions (3 mL) were assayed for galacturonic acid and neutral sugars.

A sample (2 mL of a 2 mg/mL solution) of each purified pectin was loaded on to a column (68 × 1.5 cm) of Sepharose CL-2B or CL-6B and eluted at 11 mL/h with 0.1M sodium acetate buffer (pH 4.0). Fractions (3.6 mL) were assayed for galacturonic acid and neutral sugars. The void (V_o) and total (V_t) volumes of the column were determined with a commercial apple pectin and galacturonic acid, respectively; K_{av} values of fractions were calculated as $(V_e - V_o)/(V_t - V_o)$, V_e being the elution volume of the fraction considered.

Viscosimetry. — Intrinsic viscosities (in mL/g) were obtained by measuring the flow times of solutions of pectin (2.0, 1.5, 1.0, and 0.5 mg/mL) at 30° in an Ostwald viscosimeter (flow time of solvent, 76.2 s). The solvent was 0.155M sodium chloride containing 5mM Na_2EDTA with the pH adjusted at 5. For OHP, the concentration of sodium chloride was lowered to 0.08M. Viscosity-average molecular weights were calculated according to Owens *et al.*[5].

Analysis of phenolic residues. — Phenolic residues were liberated from pectins (25 mg) by hydrolysis with 0.5M potassium hydroxide (1 mL, degassed under vacuum) for 90 min at 60° under argon[6]. The cooled solution was acidified to pH 2 with hydrochloric acid and extracted with ethyl acetate (3 × 1 mL), and the combined extracts were dried (Na_2SO_4) and concentrated under vacuum to dryness. A solution of the residue in methanol (0.1 mL) was subjected to h.p.t.l.c. on silica gel 60 (Merck), using ethyl acetate and benzene–1,4-dioxane–acetic acid (90:25:4) with sinapic acid, ferulic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, and vanillin as references. Chromatograms were developed under u.v. light (366 nm), sufficient of which, apparently, passed through the thick-walled glass trough to prevent isomer-isolation of cinnamic acid-type phenols. Detection was effected with u.v. light (366 nm) and after spraying with a freshly prepared 1:1 mixture of aqueous 1% sodium carbonate and aqueous 2% sodium permanganate.

Feruloyl groups were determined spectrophotometrically at 375 nm on freshly prepared solutions of pectin in 0.1M glycine–sodium hydroxide buffer (pH 10.0), using[7] a molar extinction coefficient of 31,600. Total phenols were determined with the Folin–Ciocalteu reagent without copper treatment, using ferulic acid as standard[8]; the sample solution (0.4 mL) was added to 0.25M Folin–Ciocalteu reagent (0.4 mL) followed, after 3 min, by M sodium carbonate (0.4 mL), and the absorbance at 750 nm was read after 1 h.

Analytical methods. — The contents of "anhydrogalacturonic acid" of pectins were determined with the automated *m*-hydroxybiphenyl method[9]. Neutral sugars in pectins (expressed as "anhydroarabinose") were determined with the automated orcinol method[10]. Corrections were made for interference from "anhydrogalacturonic acid".

For the determination of neutral sugars, each pectin (25 mg) was hydrolysed with 2M trifluoroacetic acid for 1.5 h at 120°. The released sugars were then reduced with sodium borohydride, acetylated, and determined by g.l.c.[11].

Methyl ester groups were determined by the method of Wood and Siddiqui[12], and the degrees of esterification were calculated (mol/mol × 100%) from the contents of methyl ester and "anhydrogalacturonic acid". Acetyl groups were liberated from pectins by hydrolysis with 0.1M sodium hydroxide for 1 h at room temperature. The pH was lowered to 4 with hydrochloric acid, and acetic acid was determined by g.l.c. with formic acid added to nitrogen as the carrier gas[13]. Low blank values for acetic acid were obtained by using water purified in a Milli-Q system (Millipore Corp.). Degrees of acetylation of pectins were calculated (mol/mol × 100%) from the contents of acetyl groups and "anhydrogalacturonic acid".

Nitrogen was determined by an automated micro-Kjeldahl method, and protein contents were estimated by multiplying the nitrogen content by 6.25.

RESULTS

Extraction and purification. — One-third of the alcohol-insoluble residue of sugar-beet pulp was solubilised in the four successive pectin extractions (Table I). As anticipated, the water-soluble pectin fraction (WSP) of beet pulp was small after extraction of the sugar in the factory. There was a minute oxalate-soluble fraction (OXP), and most of the pectin was extracted by acid (HP) and alkali (OHP).

The "anhydrogalacturonic acid" content of the extracts was low, but was increased considerably by purification of the pectins (Table II). No "anhydrogalacturonic acid" material was lost during purification of WSP, OXP, and HP on DEAE-cellulose (Fig. 1). However, recoveries of OHP were low because of irreversible binding to the column. During purification by copper precipitation, the method of choice for this pectin, no "anhydrogalacturonic acid" was lost. Neutral glycans, which do not bind to DEAE (Fig. 1) and which are apparently not bound covalently to the pectins, contain arabinose only. This material represented <1% of WSP and OXP, and 3.5 and 2.1% of HP and OHP, respectively. Adsorption on

TABLE I

YIELDS OF PECTINS OBTAINED FROM ALCOHOL-INSOLUBLE RESIDUE (AIR) AND THEIR CONTENTS OF "ANHYDROGALACTURONIC ACID"

	Yield		*Galacturonic acid (%)*
	% of AIR	*% of total pectin*	
Water-soluble pectin (WSP)	2.2[a]	6.4	30.8
Oxalate-soluble pectin (OXP)	0.53	1.6	49.6
Acid-soluble pectin (HP)	19.9	59.1	36.4
Alkali-soluble pectin (OHP)	11.1	32.9	40.6
Total pectin extracted	33.7	100	

[a]Percentages are on the basis of dry matter.

to DEAE-cellulose of polyphenol-like material, present in the crude pectin fractions, seriously limited the pectin-binding capacity of these columns.

Composition and properties. — The composition of the purified pectins is shown in Table II. Although the same neutral sugars were present in all four pectins, there were large differences in the relative amounts; arabinose and galactose were the major sugars. WSP contained much more arabinose and galactose than OXP. HP and OHP contained more rhamnose and fucose than WSP and OXP. Due to overlap of the g.l.c. peaks corresponding to these two sugars, they could not be quantified, but rhamnose was the major sugar.

The contents of methyl ester and acetyl groups are expressed as molar percentages of the galacturonic acid residues (Table III). The low values for OHP result from nearly complete de-esterification during extraction.

In an attempt to analyse the preparations completely (Table II), the contents of phenols[8] and proteins were determined. At pH 4.8, after purification on DEAE-cellulose, most of the carboxyl groups will have sodium counterions; the apparent pK values of pectins[14] are below pH 4.

Viscosimetric characteristics and viscosity-average molecular weights of the four pectins are given in Table IV; the ionic strength of the solvent had to be lowered to one-half, in order to keep the alkali-soluble pectin in solution so that the values for this pectin should be considered with caution.

Gel-permeation chromatograms of the purified pectins are presented in Fig.

TABLE II

COMPOSITION OF PURIFIED PECTINS FROM SUGAR-BEET PULP[a]

	Pectins			
	Water-soluble	*Oxalate-soluble*	*Acid-soluble*	*Alkali-soluble*
Galacturonic acid	54.4[b] (1.2)[c]	77.9 (1.7)	65.1 (1.0)	54.9 (1.2)
Total neutral sugars	16.5[b]	5.7	18.9	24.3
Rhamnose + fucose	0.89	0.86	2.25	3.17
Arabinose	8.44	1.85	9.97	12.49
Xylose	0.14	0.16	0.17	0.23
Mannose	0.18	0.14	0.12	0
Galactose	6.46	2.43	5.93	8.09
Glucose	0.39	0.21	0.44	0.31
Methyl ester groups	7.24	8.19	7.09	0.72
Acetyl groups	5.71	4.04	7.53	0.74
Total phenols	1.0	1.1	1.7	2.0
Feruloyl groups	0.10	0.04	0.48	0.57
Proteins (N × 6.25)	6.2	—	3.4	4.0
Sodium	1.7	4.1	3.3	6.6
Total	92.8	101.0	107.0	93.3

[a]As % of dry matter. [b]Values recorded as "anhydro" sugars. [c]Values in parentheses are standard deviations (10 determinations).

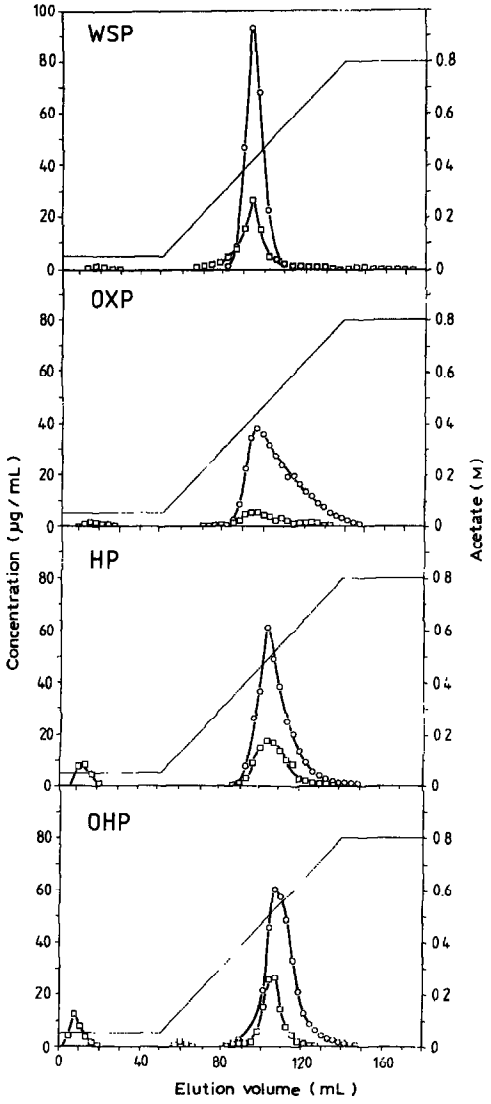

Fig. 1. Elution of crude pectins from sugar-beet pulp from DEAE-Sepharose CL-6B with sodium acetate buffer (pH 4.8) (see Experimental): —○—, "anhydrogalacturonic acid"; —□—, neutral sugars. The recoveries were 90–100%, but with OHP only 37% of "anhydrogalacturonic acid" was recovered. To make the elution profiles comparable, they were drawn for 1 mg of "anhydrogalacturonic acid" recovered for water-soluble (WSP), oxalate-soluble (OXP), acid-soluble (HP), and alkali-soluble pectin (OHP).

2. As most of the OXP was eluted from Sepharose CL-2B at K_{av} values approaching 1, a gel with smaller pores (CL-6B) was used for this pectin. Although the peak of the WSP was eluted at a K_{av} value higher than that of the peak of the HP, the first preparation had a higher viscosity-average molecular weight. This reproducible

TABLE III

DEGREES OF ESTERIFICATION OF PECTINS FROM SUGAR-BEET PULP

	Pectins			
	Water-soluble	*Oxalate-soluble*	*Acid-soluble*	*Alkali-soluble*
Methyl ester[a]	75.5	59.7	61.8	7.5
Acetyl[a]	31.3	15.5	34.5	4.0
Feruloyl[b]	0.61	0.70	2.5	2.3

[a]Expressed as mol per mol of galacturonic acid ×100%. [b]Expressed as mol per mol of neutral sugar residues ×100%.

TABLE IV

VISCOMETRIC CHARACTERISTICS AND VISCOSITY-AVERAGE MOLECULAR MASSES OF PECTINS FROM SUGAR-BEET PULP

	Pectins			
	Water-soluble	*Oxalate-soluble*	*Acid-soluble*	*Alkali-soluble*
Intrinsic viscosity (mL/g)	259	57	225	181
Huggins coefficient	0.38	0.78	0.65	0.63
Viscosity-average mol. mass	47,700	15,400	42,800	36,400

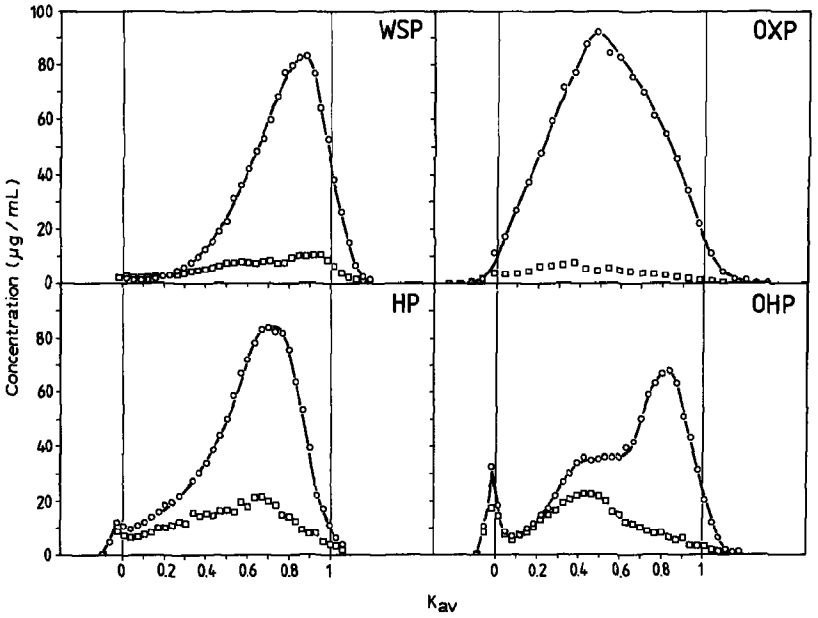

Fig. 2. Elution of purified pectins from sugar-beet pulp from Sepharose CL-2B (WSP, HP, OHP, see Fig. 1) and CL-6B (OXP) (see Experimental): —○—, "anhydrogalacturonic acid"; —□—, neutral sugars.

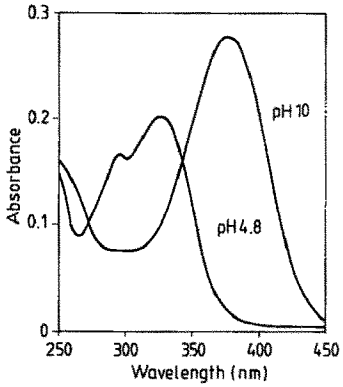

Fig. 3. Absorption spectra of alkali-soluble pectin (see Experimental).

phenomenon, observed also by Le Quère *et al.*[15] for similarly prepared pectins from sugar beet, is not readily explained.

Feruloyl groups. — Solutions of sugar-beet pectin, especially those of HP and OHP, turn yellow when the pH is raised to values of 10 or above, and there is a bathochromic shift of the double absorption peak at 300 and 325 nm at pH 4.8 towards a single peak at 375 nm at pH 10 (Fig. 3). According to Harborne[16], this bathochromic shift is indicative of esters of cinnamic acid-type phenols. After hydrolysis and extraction of liberated phenolic acids, ferulic acid was detected by h.p.t.l.c. The feruloyl content of all four pectins is given in Table II.

DISCUSSION

Extraction, purification, and composition. — Several methods exist for the extraction of pectic substances from plant material[17]. Sequential extraction is one of the more generally used methods and allows a first fractionation of the pectins; also, the hot-acid extraction step approaches the conditions used in the industrial process for pectin manufacture[2,18].

Considerably more water-soluble pectin[15] is present in fresh sugar-beet cossettes than found in pressed sugar-beet pulp, most of this fraction being lost in the leaching process. The water-soluble pectin fraction was characterised by the highest content of methyl ester groups, a fairly high content of neutral sugars, and the highest viscosity-average molecular weight.

Oxalate-soluble pectin often has a low content of methyl ester groups[17], but here it was distinguished by its low contents of acetyl groups and neutral sugars, its high content of "anhydrogalacturonic acid", and its low viscosity-average molecular weight.

The bulk of the pectin was extracted with 0.05M hydrochloric acid at 85°. The effect of a mild-acid treatment on the extractability of pectin from cell-wall material is well known, and the extraction of commercial pectin is based largely on this

phenomenon[2,18]. Some covalent linkages, especially those involving arabinose and rhamnose residues, will be broken under these conditions[19]. However, it cannot be concluded that cleavage of these linkages is the cause of the solubilisation of pectin, which may be due to hydrolysis of, as yet, unidentified linkages[20].

Subsequent treatment with alkali liberates more pectin, probably through limited β-elimination reactions of methyl galacturonate residues. Simultaneously, most of the methyl ester and acetyl groups are removed (Table III). However, more severe conditions were needed to hydrolyse the feruloyl groups. At this stage, not all of the pectin was extracted from the alcohol-insoluble residue, its content of "anhydrogalacturonic acid" being still 5%.

Acid- and alkali-soluble pectins are distinguished from the other two pectins by their high contents of rhamnose, fucose, and feruloyl groups but, although extracted through degradation, their molecular weights were not much lower than that of the water-soluble pectin.

As anticipated[21], the pectins with decreasing contents of methyl ester groups were displaced from DEAE-Sepharose by increasing concentrations of salt, and some material (pectate) was bound irreversibly (Fig. 1). Water- and acid-soluble pectins were eluted as sharp, homogeneous peaks, but oxalate-soluble pectin was eluted as a broad, tailing peak, reflecting a large variation in the contents of methyl ester groups.

Gel-filtration chromatograms of the pectin showed a fairly continuous variation of the distribution of neutral sugar residues, except for the alkali-soluble pectin. This pectin apparently consists of two populations of molecules: large, which are rich in neutral sugars, and small, with low contents of neutral sugars. This elution profile points towards cleavage of galacturonosyl linkages during alkaline extraction (see following paper[22]).

The origin of the nitrogen in the purified pectins has not been established but, since the association of proteins with pectic substances appears to be a general phenomenon[23,24], the nitrogen is expressed as protein in Table II.

Each of the four pectin fractions appears to have characteristic features. This is also true for the sugar-beet pectins as a group, compared to recently described pectins from apple[25,26], lemon[27], and cherries[28]. They resemble those of apricots[29], in such features as their contents of neutral sugars, methyl ester groups, and acetyl groups.

Phenols. — Crude pectins from sugar-beet pulp are often dark-coloured and have a high content of phenolic material. Most of this material may be eliminated by chromatography on DEAE-cellulose. However, even after purification, the pectins contain 1–2% of phenols (Table II), at least part of which (feruloyl groups) are covalently bound. Perhaps the best evidence for the presence of feruloyl groups is the fact that gels can be produced from sugar-beet pectin by an enzymic reaction in which the feruloyl residues are involved[30], namely, their dimerisation with hydrogen peroxide–peroxidase, as described by Geissmann and Neukom[6].

In the following paper[22], attention is paid to the location of these phenolic

substituents. Fry[31] showed that feruloyl groups were bound to non-reducing terminal residues of arabinose and galactose side-chains of the pectin fraction of the primary cell walls of spinach. Of the neutral sugar residues of spinach pectin, ~1.7% carry a feruloyl residue[31] [*cf.* 0.61–2.5% in sugar-beet pectins (Table III)].

Ferulic acid is bound to unlignified plant cell-wall material[32,33], a structural feature which appears to be widespread in monocotyledons[34]; in dicotyledons[35], it is restricted to the *Caryophyllales (Centrospermae)*, to which both spinach and sugar beet belong. In agreement with these data, feruloyl groups were found to be absent from pectins from other plant sources, including potato, apple, citrus, apricots, and cherry[30].

REFERENCES

1 R. M. McCready, *J. Am. Soc. Sugar Beet Technol.*, 14 (1966) 260–270.
2 W. Pilnik, A. G. J. Voragen, H. Neukom, and E. Nittner, in *Ullman's Encyclopädie der Technischen Chemie*, Vol. 19, Verlag Chemie, Weinheim, 1980, pp. 233–263.
3 E. L. Pippen, R. M. McCready, and H. S. Owens, *J. Am. Chem. Soc.*, 72 (1950) 813–816.
4 M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 250–256.
5 H. S. Owens, H. Lotzkar, T. H. Schultz, and W. D. Maclay, *J. Am. Chem. Soc.*, 68 (1946) 1628–1632.
6 T. Geissmann and H. Neukom, *Lebensm.-Wiss. Technol.*, 6 (1973) 59–62.
7 S. C. Fry, *Biochem. J.*, 203 (1982) 493–504.
8 T. Swain and W. E. Hillis, *J. Sci. Food Agric.*, 10 (1959) 63–68.
9 J. F. Thibault, *Lebensm.-Wiss. Technol.*, 12 (1979) 247–251.
10 M. T. Tollier and J. P. Robin, *Ann. Technol. Agric.*, 28 (1979) 1–15.
11 P. Albersheim, D. T. Nevins, P. D. English, and A. Karr, *Carbohydr. Res.*, 5 (1967) 340–345.
12 P. J. Wood and I. R. Siddiqui, *Anal. Biochem.*, 39 (1971) 418–428.
13 G. C. Cochrane, *J. Chromatogr. Sci.*, 13 (1975) 440–447.
14 R. Speiser, C. H. Hills, and C. R. Eddy, *J. Phys. Chem.*, 49 (1945) 328–343.
15 J. M. Le Quere, A. Baron, E. Segard, and J. F. Drilleau, *Sci. Aliments*, 1 (1981) 501–511.
16 J. B. Harborne, in J. B. Pridham (Ed.), *Methods in Polyphenol Chemistry*, Pergamon, Oxford, 1964, pp. 13–36.
17 W. Pilnik and A. G. J. Voragen, in A. C. Hulme (Ed.). *The Biochemistry of Fruits and Their Products*, Vol. 1, Academic Press, London, pp. 53–87.
18 F. Michel, J. F. Thibault, C. Mercier, F. Heitz, and F. Pouillaude, *J. Food Sci.*, 50 (1985) 1499–1502.
19 J. N. BeMiller, *Adv. Carbohydr. Chem.*, 22 (1967) 25–108.
20 I. M. Bartley, *Phytochemistry*, 15 (1976) 625–626.
21 W. H. van Deventer-Schriemer and W. Pilnik, *Lebensm.-Wiss. Technol.*, 9 (1976) 42–44.
22 F. M. Rombouts and J. F. Thibault, *Carbohydr. Res.*, 154 (1986) 189–203.
23 M. McNeil, A. G. Darvill, and P. Albersheim, *Plant Physiol.*, 70 (1982) 1586–1591.
24 B. J. H. Stevens and R. R. Selvendran, *Carbohydr. Res.*, 128 (1984) 321–333.
25 J. A. de Vries, A. G. J. Voragen, F. M. Rombouts, and W. Pilnik, *Carbohydr. Polym.*, 1 (1981) 117–127.
26 X. Rouau and J. F. Thibault, *Carbohydr. Polym.*, 4 (1984) 111–125.
27 J. A. de Vries, F. M. Rombouts, A. G. J. Voragen, and W. Pilnik, *Carbohydr. Polym.*, 4 (1984) 89–101.
28 M. Barbier and J. F. Thibault, *Phytochemistry*, 21 (1982) 111–115.
29 M. Souty, J. F. Thibault, G. Navarro-Garcia, J. M. Lopez-Roca, and L. Breuils, *Sci. Aliments*, 1 (1981) 67–80.
30 F. M. Rombouts, J. F. Thibault, and C. Mercier, Fr. Pat. 8,307,208 (1983) *Chem. Abstr.*, 102 (1985) 60792u.

31 S. C. FRY, *Planta*, 157 (1983) 111–123.
32 H. FAUSCH, W. KUENDIG, AND H. NEUKOM, *Nature (London)*, 199 (1963) 287.
33 R. D. HARTLEY, *Phytochemistry*, 12 (1973) 661–665.
34 P. J. HARRIS AND R. D. HARTLEY, *Biochem. Syst. Ecol.*, 8 (1980) 153–160.
35 R. D. HARTLEY AND P. J. HARRIS, *Biochem. Syst. Ecol.*, 8 (1980) 189–203.