ELSEVIER

Carbohydrate Research 301 (1997) 177–185

CARBOHYDRATE RESEARCH

Extraction of pectic substances from dehulled rapeseed

Ingrid Eriksson *, Roger Andersson, Per Åman

Swedish University of Agricultural Sciences, Department of Food Science, P.O. Box 7051, S-750 07 Uppsala, Sweden

Received 4 July 1996; accepted 25 January 1997

Abstract

The water-insoluble residue of dehulled, defatted, and 80% ethanol-treated rapeseed was extracted following 10 different procedures, with initial extractions, comparing the effect of extracting agent [cyclohexane-*trans*-1,2-diamine-*N,N,N',N'*-tetraacetate (CDTA) or buffer], pH (5 or 6.5), and ionic strength (0.02, 0.05 M, and in the case of buffer also 0.1 M) on yield and composition of the extracts. All 10 initial extractions were followed by two extractions with sodium carbonate at pH 10. The highest yield of pectic substances was obtained with CDTA at pH 6.5. A synergistic effect on the extracting efficiency between CDTA and pH seemed to exist. The monosaccharide composition of the polysaccharides was also different. The pectin extracted at pH 6.5 with CDTA had a higher proportion of uronic acids, suggesting a less branched variety of pectin. The sodium carbonate-extractions partly extracted a different population of pectin than CDTA. Two extracts (0.05 M CDTA/pH 6.5 and 0.02 M CDTA/pH 5) were further characterised by ion-exchange chromatography and monosaccharide analysis of the fractions. The proportion of neutral (non-retained) and acidic (gradient-eluted) polysaccharide material differed between the two extracts. The gradient-eluted material of the 0.05 M CDTA/pH 6.5 extract was retained longer on the column than the 0.02 M CDTA/pH 5 extract. © 1997 Elsevier Science Ltd.

Keywords: Pectic substances; Rapeseed

1. Introduction

Previously, different extraction procedures for the isolation of pectic substances from rapeseed meal have been described. During the seventies, Theander and Åman [1] used 2% EDTA as an extracting agent, whereas Siddiqui and Wood [2] used 2% EDTA at room temperature followed by 0.5% hot ammonium oxalate. The ammonium oxalate fraction was found to consist mainly of pectin, heavily branched with mostly arabinose and xylose units (75% of the galacturonosyl residues substituted). Aspinall and Jiang [3] also extracted pectin from rapeseed hulls with hot ammonium oxalate, from which a pectin was purified containing 76% uronic acids. Later studies of polysaccharides in rapeseed were mainly concerned with content and composition rather than characterisation of rapeseed polysaccharides. The chelating agent cy-

* Corresponding author.

PII S0008-6215(97)00096-7

clohexane-*trans*-1,2-diamine-*N,N,N',N'*-tetraacetate (CDTA) and cold sodium carbonate were shown to efficiently extract pectic substances from plant cell walls under relatively mild conditions [4,5]. These extractants have subsequently been used to extract pectic substances from a wide variety of plant materials, including onion [6], pea hulls [7], apple [8], mung bean cotyledon [9], tomato [10], and the parenchymatous tissue of runner bean [11]. Extraction with CDTA is thought to release pectic material bound by calcium ions in the cell walls, while sodium carbonate is thought to release polysaccharides attached by covalent alkali-labile cross-links, possibly phenolic esters [4]. Renard and Thibault [12] compared different extraction conditions and extracting agents (CDTA, EDTA, and buffer) on apple and sugar beet, and found large differences in yields between a pH 4.5/20 °C extraction and a pH 6.5/80 °C extraction, but little differences between the extractants.

In previous papers, we have investigated differences in polysaccharide composition in different varieties of rapeseed and between rapeseed hull and dehulled seed [13], as well as characterised a water-soluble fraction of dehulled rapeseed [14]. In this study, different conditions for the extraction of pectic substances from rapeseed meal with CDTA were compared.

2. Experimental

General.—The content of polysaccharide material in the extracts, and in the fractions prepared by EtOH precipitation, was analysed according to the Uppsala method of dietary fibre analysis [15], involving hydrolysis in 0.4 M H_2SO_4 (1 h, 125 °C), and determination of the neutral monosaccharide residues as alditol acetates by GLC and the uronic acids colorimetrically. The method was modified as described earlier [14]. The content of neutral monosaccharide residues in polysaccharide fractions obtained by ion-exchange chromatography was determined by monosaccharide analysis employing hydrolysis in 1 M CF_3CO_2H (90 min, 125 °C), reduction, and acetyla-

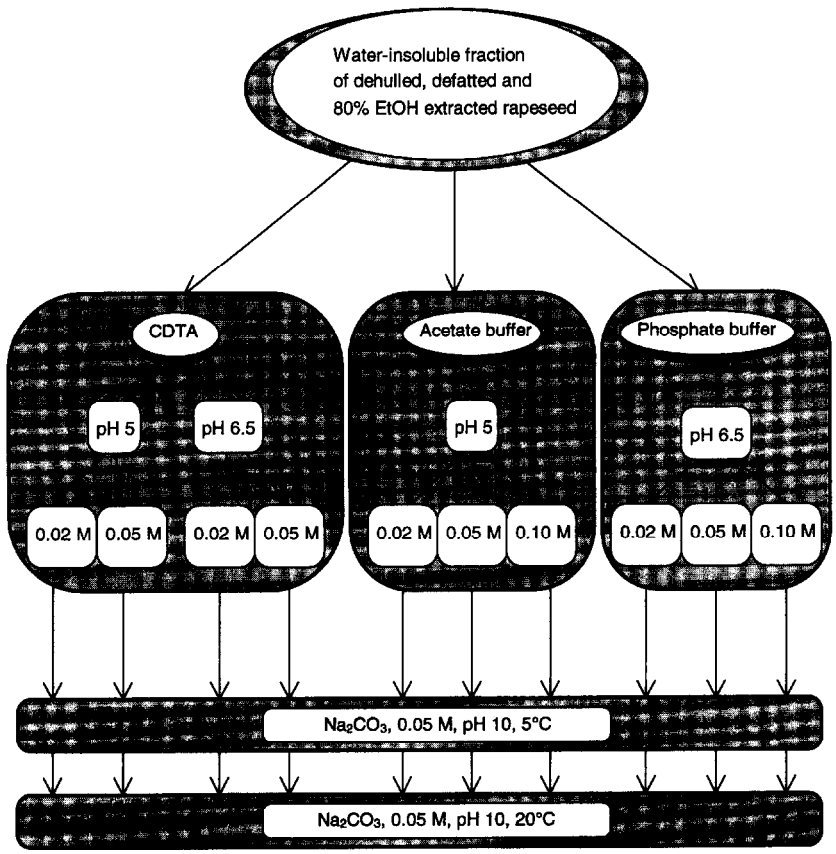

Fig. 1. Extraction scheme delineating the 10 sequences, each including three steps, investigated for the extraction of pectic substances from dehulled rapeseed meal. In the initial extraction step different extracting agents, pH, and ionic strengths were studied.

tion using 1-methylimidazole as a catalyst. The resulting alditol acetates were quantified by GLC as in the Uppsala method. Uronic acids in these fractions were determined by the method of Scott [16]. ^1H NMR spectra were recorded at 85 °C on a Varian VXR 400 instrument (400 MHz), using D_2O as solvent. CDTA was dissolved in D_2O containing NaOD.

Extraction.—Dehulled, partially defatted rapeseed meal from the winter rapeseed variety Casino was prepared according to Eriksson et al. [14]. The meal was further defatted with 2:3 2-propanol–hexane, extracted with aq 80% EtOH, and dried before being water-extracted, as described previously [14]. The isolated water-soluble fraction and the insoluble residue constituted 2.9 and ~ 90%, respectively, of defatted and 80% EtOH-extracted dehulled meal. Ten different procedures, as depicted in Fig. 1, making use of CDTA (as the sodium salt), acetate buffer at pH 5, or phosphate buffer at pH 6.5, were evaluated for isolating the pectic material from the water-insoluble residue. Residues from the CDTA- and buffer-extractions were further extracted with aq Na_2CO_3 (see Fig. 1). The extractions were performed at least in duplicate. The procedures followed were essentially part of those used for the extraction of onion cell walls [6] and of apple [8]. In more detail, the water-insoluble residue (400 mg) was extracted twice with extractant (CDTA or buffer) containing 0.02% NaN_3 and trypsin (0.125 mg/mL) (EC 3.4.21.4, bovine pancreas, Cat. No. T 8253, Sigma). These mixtures were incubated in 50-mL tubes for 5 h at 40 °C in a water bath with shaker. After cooling and centrifugation (1500 *g*, 30 min), supernatants from the two extractions were pooled, dialysed against deionised water for 72 h (Spectra/por, molecular weight cut-off 12–14,000), and freeze-dried. CDTA- or buffer-insoluble residues were washed with deionised water and freeze-dried before extraction with 0.05 M Na_2CO_3 (50 mL) containing 0.02 M $NaBH_4$ (pH 10.3) overnight at 5 °C. After centrifugation (1500 *g*, 30 min), the residue was extracted with a fresh vol of the same soln for 2 h at room temperature with stirring, before centrifugation as above. Then, the supernatants were adjusted to pH 5 with HOAc, dialysed, centrifuged as above, and freeze-dried.

Ethanol precipitation.—Ethanol precipitation was employed to remove CDTA from the 0.05 M/pH 6.5 and 0.02 M/pH 5 extracts. To this end, 100 mg of a freeze-dried sample, pooled from several extractions, was suspended in deionised water (13 mL) and stirred for 30 min. To precipitate the pectin, 95% EtOH (37 mL) was added slowly, and the tubes were stored in the refrigerator for 30 min before stirring again for 30 min. After centrifugation (1000 *g*, 20 min) and decantation of the supernatant, the pellet was washed twice with 70% EtOH (50 mL) by centrifugation (1000 *g*, 20 min), and each supernatant decanted. Supernatants were pooled, concd in a rotary evaporator, and freeze-dried. The pellet was re-suspended in water and freeze-dried. Supernatants and pellets were analysed for the content of polysaccharide material.

Ion-exchange chromatography.—The EtOH-precipitated extracts (5–10 mg) were suspended in deionised water (6 mL) and centrifuged (1000 *g*, 20 min). The supernatant was applied on a DEAE-Sephadex CL-6B column (8 × 1.6 cm) equilibrated with 0.05 M acetate buffer, pH 4.8. Neutral material was eluted with 40 mL of the equilibration buffer. Material retained on the column was eluted with a linear concn gradient from 0.05 to 2.0 M acetate buffer at pH 4.8 (80 mL) at a flow rate of 1 mL/min. Fractions of 2 mL were collected and analysed for total carbohydrate content with the phenol–H_2SO_4 assay [17], and for content of uronic acid and neutral monosaccharide residues as outlined in the General section. The column was washed with 2 M NaCl between runs according to the manufacturer's instructions.

3. Results and discussion

Yield of extracts.—The extraction scheme employed in this study (Fig. 1) was to a large extent inspired by the paper of Renard and Thibault [12], who investigated different extracting conditions on apple and sugar beet. The philosophy of the 10 extracting sequences, in the present paper, was to illustrate the importance of extracting agent, pH, and ionic strength on the extracting efficiency and composition of the extracts obtained from water-insoluble rapeseed meal. Extraction time and temperature are of course also important parameters, but they were not investigated here. A moderate temperature of 40 °C was chosen, since some pre-experiments showed that the addition of trypsin to the extraction mixture removed most of the glucose residues, probably originating from glycoproteins, from the isolated extracts. It should be noted that extraction at 20 °C gave essentially the same results as extraction at 40 °C without trypsin. Since 0.05 M CDTA at pH 6.5 [5–7,9,10] or 0.02 M CDTA at pH 5 [8] have been quite commonly used for the extraction of pectins from a

wide variety of plant materials, it was a natural choice to compare these conditions here.

Only 1% of the total polysaccharide material and 0.9% of the uronic acid residues present in the water-insoluble rapeseed meal were extracted with 0.02 M CDTA at pH 5 (Table 1). Increasing the ionic strength to 0.05 M at the same pH yielded values of 1.5 and 2.2%, respectively. However, carrying out the extraction at pH 6.5 increased the yield of polysaccharide material to 5.0% and of uronic acid residues to 13.3% for 0.02 M CDTA and to 5.4 and 13.8%, respectively, for 0.05 M CDTA. It is evident from these values that the pH is an important factor for the extraction of pectic substances from the water-insoluble rapeseed fraction, while ionic strength seems to be of less importance, at least within this commonly used range. A comparison with the corresponding values for the acetate buffer (pH 5) and the phosphate buffer (pH 6.5) extractions indicated that the presence of the chelating agent CDTA was also necessary. The yields of polysaccharide material as well as uronic acid residues were low, comparable with the values for CDTA at pH 5, regardless of the ionic strength used (0.02, 0.05, or 0.1 M). However, the pH had some effect here also; for example, the yields of polysaccharide material increased from 0.9% when using the 0.02 M acetate buffer at pH 5, to 1.7% for the phosphate buffer at pH 6.5. Yields of uronic acids increased similarly from 0.5 to 1.9%. Similar values were observed for the buffer-extractions at 0.05 and 0.1 M. It seems, therefore, that there is a synergistic effect when employing a chelating agent like CDTA and a higher pH value (6.5) in the extraction of pectic substances from rapeseed meal.

Pectin is reported to be most stable at pH 4, and both below and above this value deesterification and depolymerisation of the pectin chain occur [18]. At pH 6.5, some degradation of the pectin may be ex-

Table 1
Yields of extracts (%) prepared sequentially from the water-insoluble fraction of dehulled, defatted, and 80% EtOH-extracted rapeseed (winter rapeseed variety Casino). The extraction scheme is explained in Fig. 1

	Initial extraction at pH 5			Initial extraction at pH 6.5		
	Weight [a]	Polysaccharide material [b]	Uronic acid residues [c]	Weight [a]	Polysaccharide material [b]	Uronic acid residues [c]
CDTA 0.02 M	11.2	1.0	0.9	26.0	5.0	13.3
Na_2CO_3:1	5.4	2.8	8.1	4.7	2.0	3.7
Na_2CO_3:2	1.8	0.7	1.8	1.6	0.5	0.9
Total	18.4	4.5	10.8	32.3	7.5	17.9
CDTA 0.05 M	14.5	1.5	2.2	32.6	5.4	13.8
Na_2CO_3:1	5.4	2.5	7.5	5.2	2.5	4.9
Na_2CO_3:2	2.0	0.6	1.4	1.5	0.6	1.0
Total	21.9	4.6	11.0	39.3	8.5	19.7
Buffer 0.02 M [d]	11.9	0.9	0.5	10.2	1.7	1.9
Na_2CO_3:1	2.9	1.1	1.7	1.2	0.4	0.6
Na_2CO_3:2	1.2	0.5	1.7	0.7	0.5	1.4
Total	16.0	2.5	3.9	12.1	2.5	4.0
Buffer 0.05 M [d]	10.7	0.9	0.5	10.6	1.8	2.1
Na_2CO_3:1	3.0	1.0	1.7	2.9	0.9	1.2
Na_2CO_3:2	1.7	0.6	1.8	1.0	0.6	1.8
Total	15.4	2.6	4.0	14.4	3.3	5.1
Buffer 0.1 M [d]	12.2	1.2	0.7	9.3	1.6	1.7
Na_2CO_3:1	–	–	–	2.2	0.5	0.7
Na_2CO_3:2	–	–	–	1.1	0.5	1.8
Total	–	–	–	12.6	4.1	2.7

[a] Expressed as percent of water-insoluble fraction, DM.
[b] Expressed as percent of polysaccharide material in water-insoluble fraction.
[c] Expressed as percent of uronic acid residues in water-insoluble fraction.
[d] Acetate buffer at pH 5 and phosphate buffer at pH 6.5.

pected, which could partly explain the higher yields obtained at this pH. If some deesterification was taking place, resulting in increased ionisation of the pectin chains, a higher extracting efficiency could be expected. Possible degradation due to higher pH cannot be the only explanation, however, since the buffer-extraction at pH 6.5 did not yield as high an amount of pectin as the CDTA-extraction at the same pH. CDTA has been shown to be an effective Ca^{2+}-chelating agent [4,5], and may release pectin bound tightly with Ca^{2+}-bridges. Weightman et al. [7] stated that CDTA extracted high yields of pectin from pea hulls (18% of the uronic acids), especially compared with water-extraction (2%), because of the low methylation degree of pea hull pectin. This could also be the case for the pectin of dehulled rapeseed meal, where water-extraction extracted only 3.3% of the uronic acid residues [14]. A lower degree of methyla-

Table 2
Total polysaccharide content (% of fraction, dry weight) and relative composition (weight%) of extracts prepared sequentially from the water-insoluble fraction of dehulled, defatted, and extracted rapeseed (winter rapeseed variety Casino)

	Total polysaccharide content	Relative monosaccharide composition								Uronic acids/Rha ratio
		Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Uronic acids	
Water-insoluble residue	27.1	2.0	1.2	21.7	11.1	2.0	9.9	32.6	19.4	
CDTA 0.02 M, pH 5	2.4	1.5	1.1	31.5	8.6	6.9	18.1	14.2	18.1	11.8
Na_2CO_3:1	14.1	1.5	0.7	20.9	5.7	2.6	8.0	2.9	56.0	37.1
Na_2CO_3:2	9.6	2.0	–	23.4	6.4	4.4	9.2	2.9	51.7	26.0
CDTA 0.02 M, pH 6.5	5.2	1.9	0.8	21.2	7.2	3.7	9.5	3.6	52.1	27.2
Na_2CO_3:1	11.3	2.0	0.8	33.0	7.4	4.5	11.9	3.3	37.1	18.3
Na_2CO_3:2	8.8	2.4	–	34.1	10.9	7.1	11.7	3.7	31.2	13.1
CDTA 0.05 M, pH 5	2.7	1.7	0.8	30.7	7.2	6.2	15.8	8.3	29.1	16.9
Na_2CO_3:1	12.9	1.4	0.5	22.4	5.6	2.9	8.4	2.4	56.4	40.1
Na_2CO_3:2	8.4	1.8	–	27.3	7.7	5.1	10.0	3.0	45.2	25.2
CDTA 0.05 M, pH 6.5	4.6	2.2	0.6	23.9	7.4	3.2	9.9	3.5	49.2	22.0
Na_2CO_3:1	12.8	2.5	0.8	32.1	8.0	4.1	10.8	2.7	39.0	15.7
Na_2CO_3:2	11.7	3.0	–	34.7	9.8	5.9	11.4	4.5	30.7	10.1
Buffer 0.02 M, pH 5	2.0	1.8	1.5	31.6	8.8	10.6	22.7	11.2	11.8	6.7
Na_2CO_3:1	10.0	1.8	1.4	36.0	5.9	5.9	15.0	3.6	30.5	17.4
Na_2CO_3:2	12.3	2.1	–	19.4	6.1	3.7	5.9	2.2	60.6	28.8
Buffer 0.02 M, pH 6.5	4.6	1.9	1.7	31.5	7.6	8.8	17.7	10.7	21.9	11.7
Na_2CO_3:1	8.2	1.6	1.9	31.8	6.6	6.5	14.0	3.8	34.8	22.0
Na_2CO_3:2	17.1	1.8	–	18.8	7.0	4.1	6.3	2.3	59.7	33.9
Buffer 0.05 M, pH 5	2.4	1.8	1.5	32.2	8.2	10.4	23.3	11.8	11.0	6.2
Na_2CO_3:1	9.4	1.3	0.8	35.3	6.7	5.6	14.8	4.1	31.4	24.4
Na_2CO_3:2	9.6	2.1	–	20.0	5.7	4.9	7.3	3.0	56.9	26.6
Buffer 0.05 M, pH 6.5	4.8	1.8	1.9	31.8	6.8	9.0	18.3	10.2	22.0	12.2
Na_2CO_3:1	8.2	1.4	0.9	38.9	7.2	6.6	14.8	3.4	26.9	19.4
Na_2CO_3:2	16.7	2.5	–	17.2	6.0	4.3	7.1	4.0	58.9	23.6
Buffer 0.1 M, pH 5	2.6	1.5	1.5	34.0	7.7	9.5	23.6	10.6	11.6	7.5
Na_2CO_3:1		–	–	–	–	–	–	–	–	
Na_2CO_3:2	9.6	2.1	–	19.9	6.3	4.6	7.4	4.3	55.2	25.7
Buffer 0.1 M, pH 6.5	4.9	1.5	1.4	34.7	7.1	8.2	19.0	9.1	20.6	13.6
Na_2CO_3:1	6.4	1.7	0.8	39.4	7.1	6.9	13.4	4.8	25.8	15.3
Na_2CO_3:2	13.8	1.4	–	17.6	5.7	3.8	6.4	2.5	62.7	46.0

tion could allow more Ca^{2+}-bridges and make the presence of a strong chelating agent more important. The pectin of CDTA-extracts has been found to be highly methylated in other cases, such as in apples [8]. This could explain why for apple and sugar beet the presence of CDTA in the extracting medium did not increase the yield of pectic substances to any greater degree compared to use of buffer alone [12]. Another possibly important factor could be that the higher pH increased the ionisation of the carboxylic groups of the pectin chains, thus facilitating extraction.

The yields obtained for the Na_2CO_3-extractions following the CDTA- and buffer-extractions (Table 1) illustrate that the presence of CDTA in the initial extraction facilitated the Na_2CO_3-extractions. The yield of polysaccharide material for the first Na_2CO_3-extraction was 0.4–1.1% for the six extraction series starting with buffer and 2.0–2.8% for the four starting with CDTA. Corresponding values for the yield of uronic acids were 0.6–1.7% and 3.7–8.1%, respectively. The total yields of polysaccharide material and uronic acid residues for the three extractions were 7.5–8.5% and 17.9–19.7%, respectively, for the two CDTA/pH6.5 series compared to 4.5–4.6% and 10.8–11.0% for the two CDTA/pH5 extractions. In other words, the Na_2CO_3-extractions failed to compensate for the lower recovery in the initial extraction.

Composition of extracts.—The content of total polysaccharide material in the water-insoluble residue was 27.1%, of which the major components were glucose (32.6%), uronic acids (19.4%), and arabinose (21.7%) (Table 2). The polysaccharide contents of extracts were generally low, the remainder most likely consisting of CDTA and protein. That different populations of pectin were extracted, at least partly, with the different extracting agents was supported by the values for the relative composition of polysaccharide material in the extracts. Again, it was evident that the values largely depended on whether CDTA was included in the first extraction and on the pH used. It is also interesting to study the ratio of uronic acid to rhamnose residues. With 0.02 M CDTA at pH5, uronic acids constituted 18.1% of the extracted polysaccharide material, which contained relatively high amounts of arabinose (31.5%) and galactose (18.1%). The uronic acid/rhamnose ratio was 11.8, indicating that this extract largely contained pectin of the more branched variety, which has been referred to as 'hairy' regions, as recently reviewed by Voragen et al. [19]. The following Na_2CO_3-extractions exhibited higher relative amounts of uronic acid residues and uronic acid/rhamnose ratios of 37.1 and 26.0, suggesting that these fractions contained pectic substances of the less branched variety, also referred to as 'smooth' regions. With 0.02 M CDTA at pH6.5 uronic acid residues constituted 52.1% of the polysaccharide material in the extract, and the uronic acid/rhamnose ratio was 27.2, which indicates that in this case the CDTA extracted more of the less branched type of pectin, probably in addition to the material that could be extracted with CDTA at pH5. Apparently, the higher pH was needed to extract more pectin of the less branched type. In the Na_2CO_3-extractions following this CDTA-extraction, the uronic acid/rhamnose ratio successively drops to 18.3 and 13.1. This could mean that the pectic polysaccharide population extracted with 0.02 M CDTA/pH6.5 was partly found in the first Na_2CO_3 fraction after the 0.02 M CDTA/pH5 extraction. Results for the two 0.05 M CDTA-extraction sequences were similar to those for 0.02 M CDTA, again confirming that the pH of the initial CDTA-extraction was more important than the ionic strength in determining the type of pectin extracted. That the pectic polysaccharides extracted with CDTA (0.05 M, pH6.5) were less branched than those extracted by Na_2CO_3 has also been found in earlier studies involving similar extractions of onion tissue [6], tomato [10], and mung bean cotyledons [9]. The six buffer-extraction series all exhibited comparatively low uronic acid/rhamnose ratios (6.2–13.6) for the initial buffer-extraction, with the higher values found for the pH6.5 extractions. The subsequent Na_2CO_3-extracts contained relatively higher amounts of uronic acid residues and had higher uronic acid/rhamnose ratios, comparable to the Na_2CO_3-extracts in the CDTA/pH5 extraction sequences. Again, these results stress the interaction effect of pH and the presence of CDTA in the initial extraction of the sequence. It is also notable that the CDTA-extractions at pH6.5, whether at 0.02 or 0.05 M, seemed to extract more of the less branched variety of pectin, in contrast to the other CDTA-extractions and the buffer-extractions. This could perhaps be explained if the less branched pectin was more tightly bound because of more potential binding sites for calcium ions, but other factors may also be of importance.

Ethanol precipitation.—Mort et al. [20] reported that CDTA is not easily removed by dialysis from polysaccharide fractions extracted with CDTA. The ethanol precipitation procedure used in the present study was employed in order to remove any CDTA

remaining in the extracts after extensive dialysis. The ^1H NMR spectrum of the 0.05 M CDTA/pH 6.5 extract revealed sharp unknown peaks in addition to those recognisable as belonging to polysaccharide components. This spectrum was compared with spectra run for the ethanol precipitate of the extract and pure CDTA dissolved in D_2O with added deuterated NaOH. The shifts for the unknown peaks found for the extract did not correspond to those of the commercial CDTA preparation, although the peaks were of approximately the same magnitude. In the spectrum for the ethanol precipitate of the extract these unknown peaks were not detected. We did not investigate this further since the main purpose was to discover whether the ethanol precipitation procedure was successful in removing CDTA from the extract. However, the fact that the shifts of the unknown peaks in the extract did not correspond to those of pure CDTA suggests that some interaction between the chelating agent and the polysaccharides, perhaps over a Ca^{2+}-bridge, existed. Other authors have commented on the possibility of complexing between the carboxyl groups of pectin and CDTA via calcium ions [9], and that the extent of this interaction seems to vary with the type of pectin extracted. The 70% ethanol precipitate of the 0.05 M CDTA/pH 6.5 extract contained 43% of polysaccharide material, which was much higher than the 4.6% found in the original extract. The relative composition was still similar, however, with uronic acids constituting 50.3% of the polysaccharide material and a uronic acid/rhamnose ratio of 19.5. Corresponding values for the original extract were 49.2% and 22.0, respectively. The ethanol–supernatant fraction contained very little polysaccharide material (1.7% of the fraction), of which the majority (89%) was composed of neutral monosaccharide residues. These values together with the NMR results strongly suggest that the ethanol-treatment succeeded in removing a significant part of the non-carbohydrate material from the extract.

Ion-exchange chromatography.—The 0.05 M CDTA/pH 6.5 and 0.02 M CDTA/pH 5 extracts were treated by the above-mentioned ethanol precipitation procedure and the resulting freeze-dried pellets were suspended in deionised water. However, about one-third of the ethanol precipitate, containing 24% of its sugars, in the case of the 0.05 M/pH 6.5 extract, was not soluble and was removed by centrifugation. The uronic acid/rhamnose ratio for this pellet was quite high (32.4), indicating the presence of pectin of the less branched variety. The soluble material was subjected to ion-exchange chromatography. Recovery of polysaccharides from the column was approximately two-thirds for the 0.05 M/pH 6.5 ex-

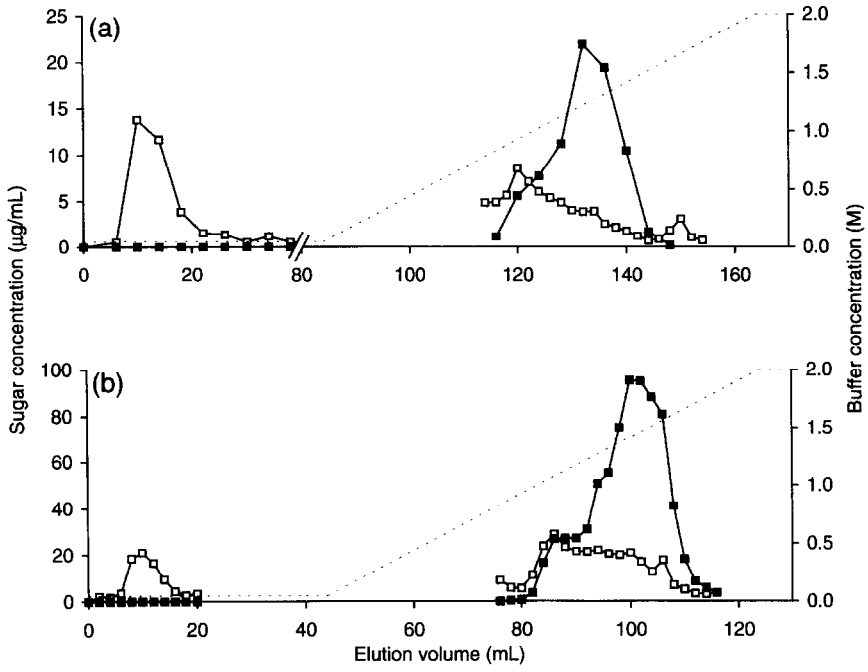

Fig. 2. Ion-exchange chromatograms obtained on a DEAE-Sephadex CL-6B column using 0.05 M acetate buffer at pH 4.8 as starting buffer. A linear concentration gradient from 0.05 to 2.0 M of the buffer was employed to elute the material retained on the column. (a) Extract obtained with 0.02 M CDTA at pH 5. (b) Extract obtained with 0.05 M CDTA at pH 6.5. ■, Uronic acids; □, neutral sugars; - - -, gradient.

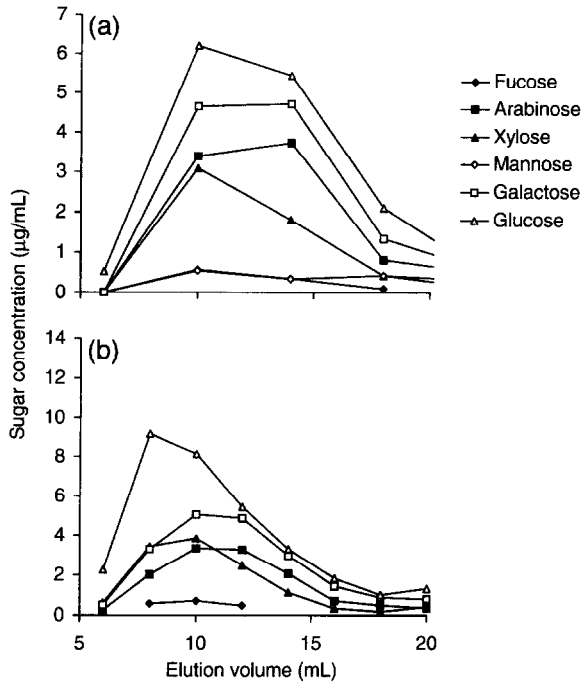

Fig. 3. Content of neutral sugar residues over the non-retained peaks in the ion-exchange chromatogram shown in Fig. 2. (a) Extract obtained with 0.02 M CDTA at pH 5. (b) Extract obtained with 0.05 M CDTA at pH 6.5.

tract. When comparing the neutral polysaccharide peaks eluted with the starting buffer in the two runs, it can be seen that the 0.02 M/pH 5 extract contained a much higher proportion of neutral monosaccharide residues not connected with pectin (Fig. 2). The elution profiles also show that in both runs, after starting the gradient, there is a population of pectin with high amounts of neutral monosaccharide residues eluting first. It is also evident that the pectin from the 0.05 M/pH 6.5 extract was eluted later than the 0.02 M/pH 5 extract in the gradient. The retention shift indicates that the 0.05 M/pH 6.5 extract included a population of pectin characterised by a lower degree of esterification than for the 0.02 M/pH 5 extract, since the ratios of neutral monosaccharide residues to uronic acid residues in the two profiles were similar. This could be due in part to chemical deesterification of the 0.05 M/pH 6.5 fraction as discussed in the section on yield of extracts.

The neutral peaks not retained on the column consisted of arabinose and galactose residues, indicating the presence of arabinogalactans, and glucose, xylose, and fucose residues, indicating the presence of xyloglucans (Fig. 3). These polysaccharides have previously been isolated from rapeseed and structurally investigated [2]. The neutral sugar analysis of the gradient-eluted peaks shows, in both profiles, that arabinose and galactose residues dominated, especially in the beginning of the peak (Fig. 4). This indicates that this pectic fraction, present for both extracts, contained a high amount of arabinogalactan side-chains. The elution profiles show that the amounts of arabinose and galactose residues gradually dropped relative to the other neutral sugars as the elution progressed. However, the arabinose/galactose ratio increased to reach a broad maximum, especially for the 0.05 M/pH 6.5 extract, indicating the presence of arabinan side-chains and a varying pectin structure over the peak. For both chromatograms, there were also amounts of xylose, glucose, and rhamnose residues in the gradient-eluted material. The presence of xylose could mean that the pectin isolated also contained xylogalacturonan material. Siddiqui and Wood isolated a pectin from rapeseed containing high amounts of xylose residues [2]. Galacturonans rich in xylose have also been found in

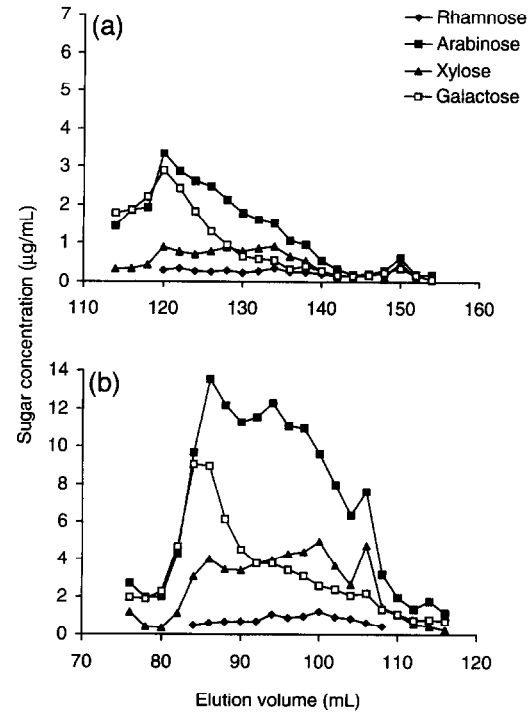

Fig. 4. Content of neutral sugar residues over the gradient-eluted peaks in the ion-exchange chromatogram shown in Fig. 2. (a) Extract obtained with 0.02 M CDTA at pH 5. (b) Extract obtained with 0.05 M CDTA at pH 6.5.

mountain pine pollen [21], soybeans [22], and apples [23]. An attempt was made to further characterise by NMR spectroscopy the fractions obtained by ion-exchange chromatography, especially in order to determine the degree of methylation. Unfortunately, it was impossible to dissolve the fractions with any reasonably mild treatments.

To conclude, both pH and the presence of CDTA were important factors for the yield and composition of pectin-extracts from water-insoluble dehulled rapeseed meal. The ionic strength of CDTA or buffer soln was found to be considerably less important. The extra polysaccharide material extracted with CDTA at pH 6.5, compared to the other extractions, seemed to consist of pectin of the less branched variety. The subsequent Na_2CO_3-treatments mainly extracted a different population of pectin compared to the CDTA-extracts. The 70% ethanol precipitation treatment of the extracts removed non-carbohydrate material (including CDTA) from the extracts to a large extent, as was shown by ^1H NMR and sugar analysis. The NMR spectra also indicated that there was some complexing of the CDTA with the pectin. Ion-exchange chromatography of two of the CDTA-extracts showed that the 0.02 M/pH 5 extract contained a higher proportion of neutral monosaccharide residues not associated with pectin than the 0.05 M/pH 6.5 extract. Additionally, the results showed that populations of pectin with variable amounts and types of neutral sugar residues such as arabinose, galactose, and xylose were present in both CDTA-extracts studied. Since pectin is one of the major cell-wall polysaccharides in rapeseed, information about the structure and properties of the pectin could be of importance for the technological and nutritional use of rapeseed products.

Acknowledgements

The authors thank Mr. Rolf Andersson for running the NMR spectra. Financial support was provided by the Swedish Council for Forestry and Agricultural Research, in collaboration with the Swedish Oil Extraction Ltd. and the Foundation of Swedish Oilseed Research/ Association of Seed and Oilseed growers.

References

[1] O. Theander and P. Åman, *Swed. J. Agric. Res.*, 7 (1977) 69–77.
[2] I.R. Siddiqui and P.J. Wood, *J. Sci. Food Agric.*, 28 (1977) 530–538.
[3] G.O. Aspinall and K.-S Jiang, *Carbohydr. Res.*, 38 (1974) 247–255.
[4] M.C. Jarvis, M.A. Hall, D.R. Threlfall, and J. Friend, *Planta*, 152 (1981) 93–100.
[5] M.C. Jarvis, *Planta*, 154 (1982) 344–346.
[6] R.J. Redgwell and R.R. Selvendran, *Carbohydr. Res.*, 157 (1986) 183–199.
[7] R.M. Weightman, C.M.G.C. Renard, and J.-F. Thibault, *Carbohydr. Polym.*, 24 (1994) 139–148.
[8] C.M.G.C. Renard, A.G.J. Voragen, J.-F. Thibault, and W. Pilnik, *Carbohydr. Polym.*, 12 (1990) 9–25.
[9] J. Gooneratne, P.W. Needs, P. Ryden, and R.R. Selvendran, *Carbohydr. Res.*, 265 (1994) 61–77.
[10] G.B. Seymour, I.J. Colquhoun, M.S. DuPont, K.R. Parsley, and R.R. Selvendran, *Phytochemistry*, 29 (1990) 725–731.
[11] P. Ryden and R.R. Selvendran, *Biochem. J.*, 269 (1990) 393–402.
[12] C.M.G.C. Renard and J.-F. Thibault, *Carbohydr. Res.*, 244 (1993) 99–114.
[13] I. Eriksson, E. Westerlund, and P. Åman, *J. Sci. Food Agric.*, 66 (1994) 233–240.
[14] I. Eriksson, R. Andersson, E. Westerlund, R. Andersson, and P. Åman, *Carbohydr. Res.*, 281 (1996) 161–172.
[15] O. Theander, P. Åman, E. Westerlund, R. Andersson, and D. Pettersson, *AOAC method 994.13, Official Methods of Analysis*, 16th ed., 1995, 1st supplement.
[16] R.W. Scott, *Anal. Chem.*, 51 (1979) 936–941.
[17] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith, *Anal. Chem.*, 3 (1956) 350–356.
[18] A.K. Andersen, *Avestring av pektin med pektinesterase: Høyfelts ^1H-NMR-spektroskopi for undersøkelse av pektinets struktur och esterasens virkemåte*, Thesis, University of Trondheim, Norway, 1990.
[19] A.G.J. Voragen, W. Pilnik, J.-F. Thibault, M.A.V. Axelos, and C.M.G.C. Renard, in A. M. Stephen (Ed.), *Food polysaccharides and their applications*, Dekker, New York, 1995, pp 287–339.
[20] A.J. Mort, B.M. Moerschbacher, M.L. Pierce, and N.O. Maness, *Carbohydr. Res.*, 215 (1991) 219–227.
[21] H.D. Bouveng, *Acta Chem. Scand.*, 19 (1965) 953–963.
[22] G.O. Aspinall, R. Begbie, A. Hamilton, and J.N.C. Whyte, *J. Chem. Soc., Chem. Commun.*, (1967) 1065–1070.
[23] H.A. Schols, E.J. Bakx, D. Schipper, and A.G.J. Voragen, *Carbohydr. Res.*, 279 (1995) 265–279.