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Cell Wall Polysaccharides from Chalkumra (*Benincasa hispida*) Fruit. Part I. Isolation and Characterization of Pectins

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Pectic polysaccharides were obtained from chalkumra (*Benincasa hispida*) fruit by sequential extraction with ammonium oxalate (fraction BOX), dilute acid (fraction BHCl), and cold dilute alkali (fraction BOH). The highest yield of polysaccharides was obtained with oxalate and HCl. BOX was enriched in partly methyl-esterified galacturonic acid, whereas BHCl and BOH contained mostly galactose. All of the extracts showed similar elution patterns in size exclusion chromatography although the intrinsic viscosities (η) were different (132 ± 6, 100 ± 5, and 285 + 10 mL/g for BOX, BHCl, and BOH, respectively). From fractionation by anion exchange chromatography, homogalacturonan (as seen from sugar analysis and Fourier transform infrared spectrum) accounted for more than half of BOX and 11% of BHCl. Methylation analyses and hydrolysis of BHCl with endo-β-(1→4)-D-galactanase showed the presence of β-(1→4)-D-galactan. The neutral galactan represented more than 76% of BHCl and ∼40% of BOH. The other polysaccharides were complex galactans in BOH and an acidic arabinan (<1%) in BOX and BHCl.

KEYWORDS: ***Benincasa hispida*; fruit; homogalacturonan; galactan; arabinan; chromatography; infrared spectroscopy; viscosity**

INTRODUCTION

Benincasa hispida (Thunb) Cogn. locally known as "chalkumra" grows in almost every part of India (*1*). The mature fruit is the primary harvested plant part, although seeds are sometimes extracted, fried, and eaten. The average production of the fruit of this green vegetable is ∼18.5 tons/hectare (*2*). Effects of rootstock on the yield and quality of *B. hispida* fruit have been assessed (*3*). Occasionally, the overproduction of this fruit creates economical problem. Means to add value to this production would contribute to lower the economic burden on communities cultivating it. *Benincasa* fruits are potential sources of dietary fibers, which account for about 27.5% of the dry weight of this vegetable (*4*). The fruit juice of *B. hispida* has shown antiinflammatory and antiulcer activities (*5*−*7*). To develop better use of this fruit, knowledge of its chemistry is necessary. The fruit contains amino acids, proteins (*8*), enzymes (*9*−*11*), vitamins (B1 and C), sterols, flavonoid C-glycoside, rare terpene (*12*, *13*), phenolic acids, free sugars such as glucose, rhamnose, and mannitol, and even some trace metals (*2*). Furthermore, the alcohol insoluble residues from fruit possess large amounts of polysaccharides (*14*), particularly pectic substances, but no detailed study on the families of polysaccharides presented therein has yet been carried out. Because pectin is a major cell wall polysaccharide in *B. hispida* fruit, information about the yield, structure, and properties of this polymer could be of importance from the industrial as well as scientific points of view.

Pectins are widely used in food, pharmaceutical, and cosmetic industries as gelling, thickening, and stabilizing agents (*15*). Changes in the texture of fruits and vegetables and in the properties of their products are related to changes in the structure of the pectic substances. The main structural feature of pectins is the linear α-(1→4)-linked D-galactopyranosyluronic acid backbone (smooth region) in which some of the carboxyl groups are in the methyl ester form (*15*, *16*). Some of the hydroxyl groups can be acetylated on O-2 and/or O-3 (*17*). The regular conformation of the poly-D-galacturonate backbone is interspersed by the covalent insertion of (1→2)-linked L-rhamnosyl residues. Other NSs such as Ara, Gal, Xyl, etc. are found in side chains attached to C-4 of some rhamnose residues and C-2 or C-3 of galacturonic acid. The number and distribution (random or blockwise) of the ester groups along the molecule play an important role on solubility, gelation, and thickening properties of pectins (*18*, *19*). Moreover, the gelling properties of pectins are influenced by the length of their side chains (*20*). The major sources of commercial pectin are apple pomaces or citrius peels, but alternative sources such as chalkumra, sugar beet, carrot, and potato may be found.

In the present work, we report the isolation of pectic polysaccharides from chalkumra fruit with maximal yield, as used on an industrial scale. Some physicochemical characteriza-

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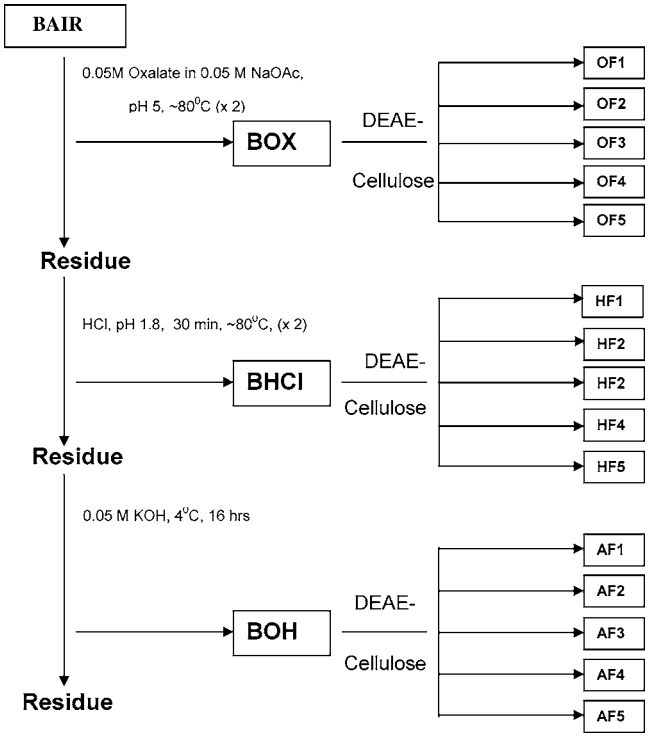

Figure 1. Scheme for the isolation and fractionation of pectic polysaccharides from the BAIR.

tions of the isolated polysaccharides are also described in order to hypothesize on their putative valorization.

MATERIALS AND METHODS

Materials. BAIRs were prepared as described previously (*14*). The fruits were collected from the local Market of Burdwan, West Bengal, India.

Isolation of Pectic Substances from BAIR. BAIR (3 g) was extracted sequentially (**Figure 1**) with (i) 0.05 M ammonium oxalate in 0.05 M NaOAc buffer (pH 5, 80 °C, 2 × 1 h, 250 mL) (BOX); (ii) HCl (pH 1.8, 80 °C, 2 × 0.5 h, 200 mL) and then washed with water (named BHCl); and (iii) 0.05 M KOH + 20 mM $NaBH_4$ (250 mL, 4 °C, 16 h), using low temperature and $NaBH_4$ to prevent alkaline peeling from the reducing end of polysaccharide chains (the extracted material was designated as BOH). After each extraction, solubilized material was separated from the insoluble residue by centrifugation (10 000*g*, 15 min), and the supernatant was filtered through a glass filter (G 3). BHCl extract was brought to pH 5 with 1 M sodium hydroxide. The alkaline solution was immediately acidified to pH 5 with dropwise addition of acetic acid over an ice bath. One drop of iso-octanol was used as an antifoaming agent. All of the extracts were concentrated, dialyzed against water, and stored as a frozen solution. To determine the yield, a part of the retentate was diluted with 4 volumes of ethanol and the recovered pellet was dried in a vacuum after solvent exchange. Two independent experiments had been carried out.

Analytical Methods. All experiments were conducted at least in duplicate, and the mean and standard deviation were directly calculated from the functions present in the Excel program. Evaporations were carried out under reduced pressure at around 50 °C. Dialysis against distilled water was performed with continuous stirring; toluene was added to inhibit microbial growth. Moisture was determined by drying ground material in an air-circulated oven at 110 °C for 3 h.

Sugar Analysis. NSs were estimated as anhydroglucose by the phenol−sulfuric acid assay (*21*). Depending on sugar composition, the evaluation was corrected afterward. Corrections were also made for interference from galacturonic acid. Total UAs were assayed as anhydrogalacturonic acid using *m*-phenyl phenol color reagent (*22*). All fractions were hydrolyzed in 2 M sulfuric acid (3 h, 100 °C) for measurement of individual NS. Sugars were reduced, acetylated, and analyzed as their alditol acetate by GLC (*23*) on columns of 3% SP-2340 on Supelcoport 100−120 mesh and DB-225 (JW) and by GLC/MS (Shimadzu QP 5050A). Myoinositol was used as an internal standard. Sugars in the acid hydrolysate were also analyzed by thin-layer chromatography as described (*24*). Alternatively, BAIR was hydrolyzed with 2 M trifluoro acetic acid (2 h at 100 °C) for soluble substances. For water insoluble residues, this hydrolysis was followed by a treatment of the residue with 72% (w/w) H_2SO_4 for 1 h at room temperature and then with 2 M H_2SO_4 for 2 h at 100 °C. Manitol and/or myoinositol were used as internal standard. The monosaccharide residues present in the hydrolysate were treated with dry 2 M methanolic HCl for 18 h at 80 °C, and the methylglycosides formed were converted into their trimethyl silyl derivatives and analyzed by GLC.

Linkage Analysis. Methylation was carried out by the method of Blakeney and Stone (*25*). The permethylated polysaccharide was hydrolyzed with 2.5 M trifloro acetic acid at 120 °C for 75 min, reduced with 1 M $NaBD_4$ in 2 M NH_4OH for 3 h at room temperature, and acetylated using perchloric acid as a catalyst. The partially methylated alditol acetates (PMAA) were analyzed by GLC and GLC/MS using DB-225 (JW) column. The temperature program was 170 °C for 15 min, 170−210 °C at 5 °C/min, and 210 °C for 15 min. The mass spectra were recorded with Shimadzu QP 5050A GLC/MS instrument at 70 eV. The PMAAs were identified as described (*26*, *27*).

Degree of Esterification. Ester-linked methanol and acetyl residues were saponified with 0.2 M NaOH/2-propanol for 3 h at 25 °C. The liberated methanol and acetic acid were determined by high-performance liquid chromatography (HPLC) (*28*). The DM and DA are defined as the number of moles of methanol and acetyl residues per hundred moles of galacturonic acid. DM was also checked by FT-IR (*29*).

Amino Acid Analysis. Protein was measured in the soluble material by the micro-Bradford method using bovin serum albumin as standard and was detected in some chromatography fractions by the absorbance at 280 nm. Amino acids were released by hydrolysis with 6 M HCl at 110 °C for 22 h in a sealed tube. Protections were done for cysteine, methionine, and tyrosine using proper protecting reagents. The liberated amino acids were analyzed by Pharmacia LKB ALPHA PLUS amino acid analyzer.

IR Spectroscopy. IR spectra were obtained on a FT-IR spectrophotometer (JASCO FT-IR-420) using KBr disks containing finely ground samples. The analyses were conducted five times.

Viscosity Measurement. To determine the intrinsic viscosity (η) of pectin, the flow time of the pectin solutions at different concentrations was determined at 30 °C using an Ubbelohde type viscometer. All flow times were averages of at least three replicates. The reduced viscosity (η_{sp}/C) was then plotted against the polymer concentration (C in g/mL), and the intrinsic viscosity was derived from extrapolation to $C = 0$.

SEC. Solutions (1−2 mL) of different extracts in 500 mM sodium acetate buffer (pH 4.0) were loaded to a column of (50 cm × 2.3 cm) Sephadex G-200 equilibrated with the same buffer. The column was eluted ascendingly with the same buffer at 20 mL h^{-1}, and the temperature was 30−35 °C. Elution of polysaccharide was expressed as a function of the partition coefficient K_{av} [$K_{av} = (V_e - V_0)/(V_t - V_0)$ with V_t and V_0 being the total and void volume of the column determined as the elution volume of glucose and blue dextran, respectively, and V_e is the elution volume of the sample]. The column was calibrated with standard dextrans within a Mw range of 10 000−500 000. Fractions (5 mL) were collected and analyzed for UA and NS content, using galacturonic acid and glucose, respectively, as standard. The analysis was carried out once.

Anion Exchange Chromatography. *System A.* Pilot experiments were initially conducted using a DEAE-cellulose column (25 cm × 1 cm) equilibrated with 0.05 M sodium acetate buffer, pH 4.5. After the sample was loaded, the column was washed with the same buffer (60 mL: fraction F1) and then eluted successively with 50 mL of 0.2 (fraction F2), 0.5 (fraction F3), and 1 M NaOAc buffer, pH 4.5 (fraction F4), in a stepwise manner. Residual bound polysaccharides were washed from the column with 50 mL of 0.2 M NaOH (Fraction F5). Fractions (10 mL) were collected and analyzed for their total sugar and UA contents.

System B. On the basis of the results of pilot experiments, all pectic fractions (BOX, BHCl, and BOH) were injected separately on a column of DEAE-cellulose (20 cm × 4 cm) in acetate form. Similar experiments

Table 1. Yields and Sugar Composition of the Polymers Isolated from the BAIR by Sequential Extractions with Inorganic Solvents (See Materials and Methods for Identification of Fractions)

	BAIR	TFA-BAIR	BOX	BHCl	BOH
yield[a]	100		21 ± 3	25 ± 3	13 ± 2
NS[b]	32 ± 2	24 ± 2	38 ± 2	68 ± 4	41 ± 3
GalA[b]	25 ± 1		53 ± 3	19 ± 2	11 ± 1
[η]	nd	nd	132 ± 6[d]	100 ± 5[d]	285 ± 10[d]
Rha[c]	1		2	1	1
Fuc[c]	1		tr	tr	tr
Ara[c]	4		5	1	6
Xyl[c]	8	3	2	tr	1
Man[c]	1	4	1	tr	1
Gal[c]	22	tr	33	76	69
Glc[c]	3	91	2	1	6
GalA[c]	58	2	55	21	16
GalA/Rha	45 ± 5	nd	37 ± 4	15 ± 3	15 ± 2
(Gal + Ara)/Rha	20 ± 3	nd	25 ± 3	55 ± 5	68 ± 6
DM	nd	nd	23 ± 3	<5	0

[a] Weight percentage of the BAIR dry weight. [b] Weight percentage of fraction dry weight. [c] Mol percent. [d] Intrinsic viscosity (C in ml g^{-1}). nd, not determined; tr, trace; –, not detected. TFA-BAIR: BAIR treated with 2 M TFA was submitted to Saeman's hydrolysis, releasing additional NSs estimated around 24% of the BAIR dry weight. The sugars consisted mainly of glucose residues as seen after methanolysis and silylation. The sugars released by the TFA pretreatment were about the same as the sugar released by 2 M H_2SO_4 reported in first column. Altogether, the sugars released by the pretreament and the Saeman's hydrolysis represent abound 80% of BAIR mass. Each value, which is given to its nearest whole number, was the mean of at least two replicates and the standard deviation was in the range of 5% relative to the mean.

were conducted as in system A for the three pectic fractions but using larger volumes of eluting buffer (450 mL at each step). The collected fractions (F1−F5) were concentrated and dialyzed against tap water and then distilled water, and finally, the concentrated retentates were diluted with 3 volumes of absolute ethanol. The precipitates, which were recovered by centrifugation (20 min at 8000*g*), were dehydrated by solvent exchange and finally dried, under vacuum, over P_2O_5. The experiment was carried out once.

Enzyme Hydrolysis. The galactan-rich pool (BHCl) was incubated (0.5 mg/mL in a 50 mM NaOAc buffer at 30 °C for 36 h) once with endo-β-(1→4)-D-galactanase (EC 3.2.1.89−0.1 U per 4 mg polysaccharides). One unit, U, is defined as the amount that liberates 1 μmol reducing sugar per min. The enzyme was purified from Pectinase 29 (*30*), an enzyme preparation derived from *Aspergillus niger* (Gist Brocades, The Netherlands). After incubation, the resulting digest was heated at 100 °C for 15 min to inactivate the enzyme. The change in Mw distribution and the release of oligomeric end products were studied by chromatography on Sephadex G-200 gel. HPAE-PAD chromatography of endogalactanase-generated fragments was achieved on a Dionex DX 500 system equipped with a GP 50 gradient pump and a CarboPac PA1 column. Sample (50 μL) was injected and eluted (1 mL min^{-1}) with the following NaOAc gradient in 100 mM NaOH: 0 → 5 min, zero gradient; 5 → 20 min, linear gradient of 0 → 35 mM NaOAc; 20 → 42 min, linear gradient of 35 → 150 mM NaOAc.

RESULTS AND DISCUSSION

Composition of the Cell Wall Material and of Its Pectic Polysaccharides after Sequential Extraction. The BAIR contained 57 ± 5% 2 M sulfuric acid hydrolyzable sugars of which about half were UAs (**Table 1**), identified as galacturonic acids by thin-layer chromatography. The main NS was galactose. BAIR was relatively poor in the other sugar characteristic of pectins such as rhamnose, arabinose, and fucose. After Saeman's hydrolysis, glucose was the main sugar released from BAIR, most of the glucose being of cellulosic origin.

About 21 ± 1% of BAIR and 39 ± 2% of its UAs were extracted by NH_4-oxalate (BOX). These extracted pectic polysaccharides were held in the walls by ionic cross-links, as chelating agents were able to extract calcium ions (*18*). The BOX fraction contained 91 ± 5% polysaccharides on dry weight basis and was composed mainly of galacturonic acid, most probably linked in a homogalacturonan backbone (*18*). Galactose, the second most abundant sugar, might be part of RG-I side chains, released by β-elimination, due to a relatively high temperature of the oxalate solution (*15*). The average DM was estimated to be 23 + 3% while the average DA was less than 1%. The protein content of the BOX fraction was low, and the most abundant amino acid was glycine (**Table 2**). Other abundant amino acids included alanine, aspartic acid/asparagine, glutamic acid/glutamine, and leucine.

Table 2. Amino Acid Composition (mol %) of Proteins Associated with Hot BOX of Cell Wall Material of *B. hispida* Fruit[a]

amino acid	mol %	amino acid	mol %
glycine	12	isoleucine	6
alanine	11	phenylalanine	5
asparagine/aspartic acid	11	lysine	5
glutamine/glutamic acid	10	arginine	4
leucine	9	proline	3
valine	7	tyrosine	2
serine	7	methionine	1
threonine	6	histidine	1

[a] Each value, which is given to its nearest whole number, was the mean of two replicates and the standard deviation was in the range of 6% relative to the mean.

BHCl extracted the highest amount of polysaccharides (BHCl) from BAIR. Mild acid treatment has been reported to remove large amounts of pectic substances from citrus, apple, or sugar beet pulp (*31*), probably by cleavage of the cross-links of the matrix polymers and other linkages such as hydrogen, ionic bonds, and glycosidic linkages. The polysaccharide content of the BHCl fraction was 87 ± 4%, of which the major sugar was galactose (**Table 1**). Arabinose accounted for less than 5% of the total sugars, but arabinosyl linkages are particularly labile in acidic solutions (*15*). UA residues accounted for 19 ± 2% (w/w) of BHCl and 17 + 1% of the UAs of BAIR. Their DMs and DAs were less than 5 and 1%, respectively.

Other pectic polysaccharides, probably cross-linked within the wall matrix (*32*), were extracted with 0.05 M KOH. The polysaccharide content of the alkali-extracted material (BOH) was low, the remainder most likely consisting of salts. The monosaccharide composition of BOH fraction was very similar to BHCl pectin (**Table 1**). Galacturonic and galactose together accounted for >86% of both fractions; therefore, they contained mostly pectic galactans. As compared to native polymers, some arabinan moieties might have been peeled during the previous acidic treatment.

As found in earlier studies reviewed by O'Neil et al. (*16*), the galacturonic acid/rhamnose ratio of pectins extracted with chelating agent (BOX) was much higher than the ratio in the acid (BHCl) and alkali-extracted (BOH) pectins. Therefore, the former contained a larger amount of the homogalacturonan (smooth) region than the two others. Conversely, (galactose + arabinose)/rhamnose ratio of the later two fractions was twice as high than that of the BOX fraction. Therefore, BHCl and BOH might contain larger side chains as compared to that BOX. For further characterization, these polysaccharides were analyzed by FT-IR, SEC, and viscosimetry.

FT-IR. FT-IR spectroscopy is a valuable tool for determining the bulk structural features of polysaccharides (*29*, *33*, *34*). The FT-IR spectra of various fractions are given in **Figure 2**. Spectra from BAIR were also recorded (data not shown). The broad band between 3600 and 3000 cm^{-1}, corresponding to vibrations of the hydroxylic groups, was similar in all of the spectra.

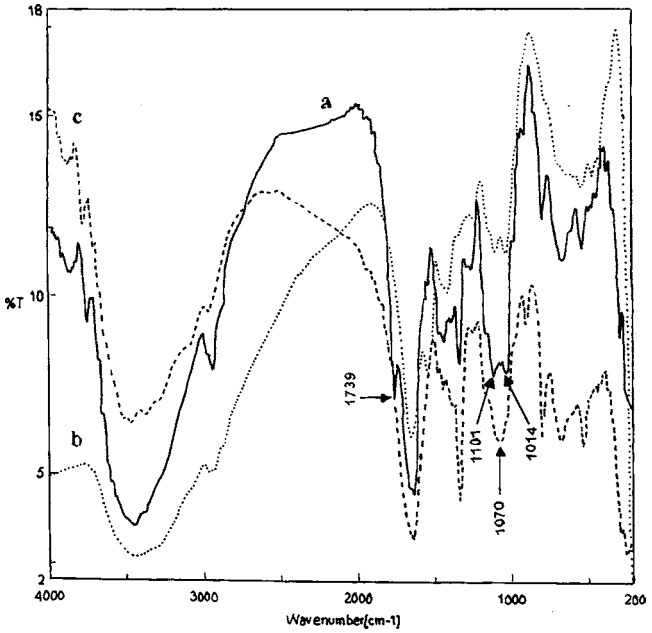

Figure 2. FT-IR spectra of polysaccharide fractions: (**a**) BOX, (**b**) de-esterified BOX, and (**c**) BHCl obtained from *B. hispida* fruit pulp. See the Materials and Methods for identification of fractions.

Methyl and methylene group vibrations around 2927 cm^{-1} were also present in the spectrum of all fractions as well as in BAIR. A band in the region 1739 cm^{-1} related to the C═O stretching of the ester group was detected in the BOX spectrum (**Figure 2a**). After saponification, the absorbance of the band at 1739 cm^{-1} disappeared (**Figure 2b**). The ratio of the peak height at 1739 cm^{-1} over the sum of the peak heights at 1739 and 1605 cm^{-1} (characteristic of ionized carboxylic acid) was varied between 0.15 and 0.4, that is around the DM value estimated by HPLC but on a larger amount of BOX pectins (25 mg/1−3 mg for FT-IR). Although not considered as strictly quantitative, the FT-IR data illustrated well the methylesterification of the BOX fraction. Besides, the BAIR spectrum also displayed similar absorption at 1605 and 1741 cm^{-1} with a ratio around 0.5. However, in BAIR, the UA ionization was not known, so it was impossible to discriminate between methylesterifed UA and UA neutralized by protons or condensed calcium. As expected, the peak height at 1739 cm^{-1} disappeared when a basic treatment was performed, due to the pectin saponification and to the neutralization of the acids by sodium ions. Structural features arising from particular conformations around the glycosidic bond of the pectins are observable in the 850−1200 cm^{-1} region (*34*). The bands at 1014 and 1101 cm^{-1}, characteristic of the UA residues in homogalacturonan (*33*, *34*), were clearly visible in the spectrum of the BOX polymer (**Figure 2a**). The IR spectra of BHCl (**Figure 2c**) and BOH (data not shown) indicated a band at 1070 cm^{-1} due to galactan and/or RG-I (*34*).

SEC. All of the extracted fractions were submitted to SEC onto Sephadex G-200 (**Figure 3**). As indicated by the K_{av} values, all fractions eluted within the fractionation range of the column. These polysaccharides were polydisperse, as generally observed for pectins. However, one major symmetrical peak accounted for 68, 53, and 60% of the fractions BOX, BHCl, and BOH, respectively, within the K_{av} ranges from 0.20 to 0.36 (BOX), 0.20 to 0.33 (BHCl), or 0.20 to 0.4 (BOH). The distribution of BHCl tailed to lower Mws while the polydispersity of BOH was much broader. On the basis of calibration with dextrans, the apparent Mw of the major peak of BOX and BHCl would be 140 000 ± 20 000 while that of BOH might be estimated to

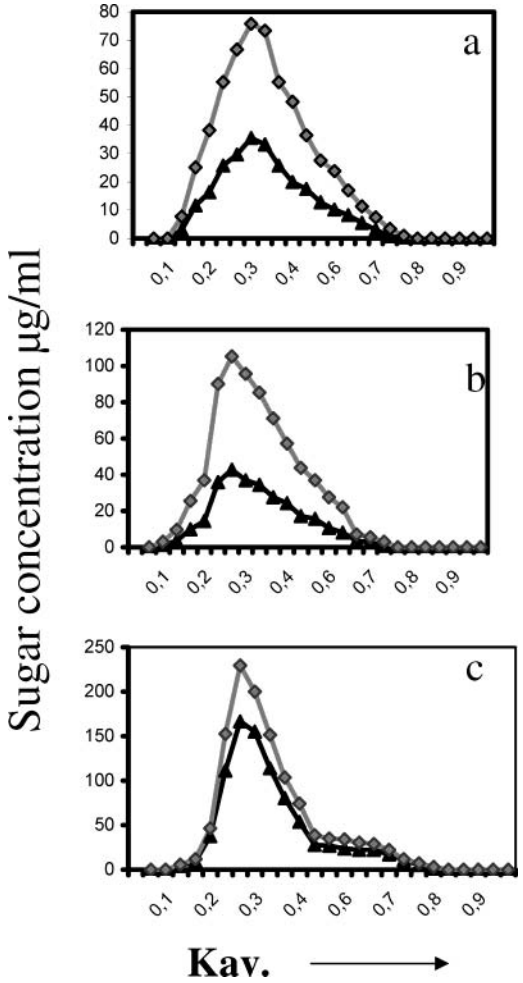

Figure 3. Elution profile of (**a**) BOX, (**b**) BHCl, and (**c**) BOH fractions on Sephadex G-200 column with 500 mM sodium acetate buffer (pH 4.0) at 20 mL/h. See text for identification of fractions.

be 120 000 ± 40 000. It should be noted, however, that polysaccharides containing UA, due to intramolecular electrostatic repulsions by charge effects, have a different hydrodynamic volume than dextrans and, therefore, elute at a different rate than expected on the basis of their Mw (*35*).

Intrinsic Viscosity. Intrinsic viscosity (η) is a measure of the hydrodynamic volume occupied by a polymer and depends on its conformation and Mw. The viscosity average Mws of polysaccharides can be determined by applying the well-known Mark−Houwink empirical equation: $(\eta) = KM^a$, K being characteristic of the primary structure of the polymer and a depending on its flexibility/rigidity. Hourdet and Muller (*36*) established two Mark−Houwink relationships for flax pectins. One of them ($a = 1.07$; $K = 0.96 \times 10^{-3}$) is suitable for linear homogalacturonan, whereas the other ($a = 0.69$; $K = 21 \times 10^{-3}$) fits well with the rhamnoglacturonan behavior, being indicative of branched and flexible polymer. Pectic fractions BOX and BHCl had intrinsic viscosities of 100 ± 5 and 132 ± 6 mL g^{-1}, which were larger than flax pectins but comparable to the values reported for apple/citrus/sugar beet pectins (*17*, *37*, *38*). From the values 0.96×10^{-3} for K and 1.07 for a, we calculated an average Mw of 63 000 and 49 000 for BOX and BHCl, respectively. However, the presence of a high amount of NSs in BHCl, which may be present as side chains of the rhamnogalacturonan (hairy) regions, might lead to higher flexibility than that of homogalacturonan and of BOX. Using values of 21×10^{-3} for K and 0.69 for a, we estimated an average Mw value of BHCl around 210 000.

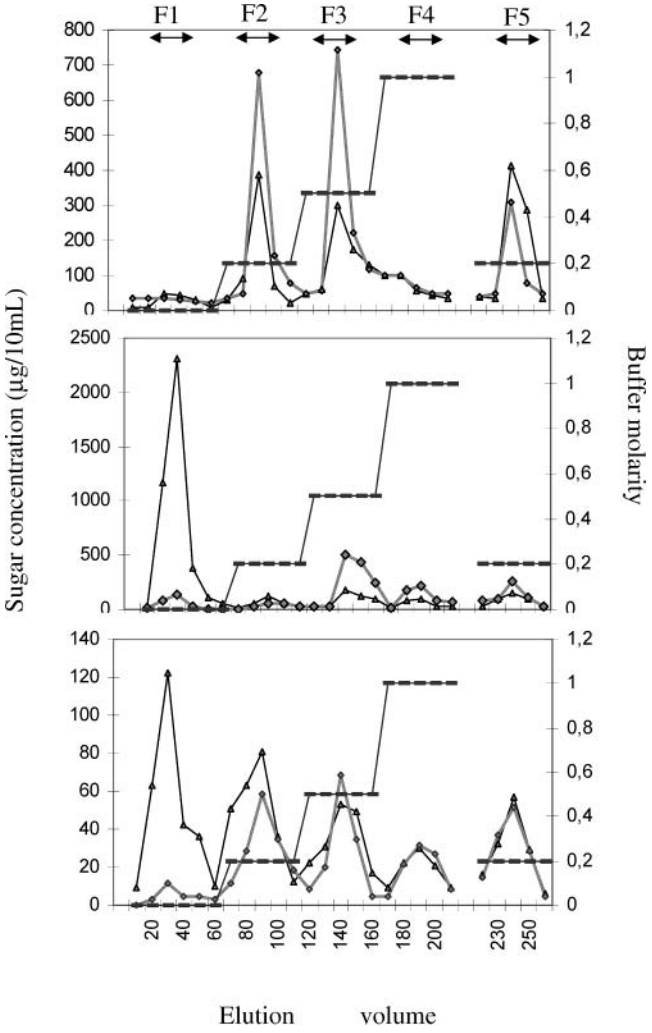

Figure 4. Anion exchange chromatography on DEAE-Cellulose column of (**a**) BOX, (**b**) BHCl, and (**c**) BOH fractions, isolated from *B. hispida* fruit. F1–4: Pooled fractions were eluted with water and NaOAc buffer (pH 4.5); the digit corresponds with the order of elution. F5: Fraction eluted with 0.2 M NaOH.

Interestingly, the intrinsic viscosity of the alkali-extracted pectin (BOH) was higher than the other two fractions (BOX and BHCl) and close to that of hop pectins (*39*). It has been well-documented that DE has an impact on the conformational and rheological properties of pectins (*19*, *20*, *40*). The alkali-extracted pectin had no methyl ester groups, and therefore, a conformational change might facilitate interactions between macromolecules and increase the viscosity. Estimation of Mw varied greatly from 130 000 to 975 000 depending on the chosen values for parameters *a* and *K*, as given in ref *36*. High-performance size chromatography, combined with a refractive index detector, viscosimeter, and right laser light scattering detector, should be developed to get better insight into the Mw and conformation of the three pectic fractions.

Anion Exchange Chromatography. Attempts were made to purify these fractions using anion exchange chromatography (**Figure 4**). The amount of polymers isolated from the columns and the sugar composition of each fraction are given in **Table 3**. Pool 1 consisted of unbound polysaccharides and, as expected, had a low amount of UAs (1−6 mol %). The amount of galacturonic acid increased with the ionic strength of the eluent. However, it was not the case in pool 5, suggesting adsorption of polymers with the anionic matrix.

From BOX, pool 1 (OF1) was obtained in low amounts and contained mainly glucose, galactose, and xylose. In the major fraction OF3, but also in OF2, galacturonic acids accounted for more than 75% of the charged fractions. They were therefore essentially homogalacturonans that might be partially methyl-esterified, as indicated by their early elution. On the other hand, OF5, also enriched in galacturonic acid, might contain non-methylesterified homogalacturonan. It enclosed also galactose, xylose, and glucose, as in OF1. Xylose has been reported in some xylogalactacturonans (*41*, *42*). Xyloglucan, a family of neutral polysaccharides that can be galactosylated, in some cases might be covalently linked to pectic components (*32*). SEC of OF3 (K_{av} < 0.28) and OF5 (K_{av} range, 0.25−0.48) indicated a relationship between K_{av} and the charge density of the polymer (related to the ionic strength of the DEAE eluting buffer). The hydrodynamic volume of homogalacturonan should increase with its charge. The K_{av} of OF5 was higher than that of OF3, and the Mw of OF5 was significantly smaller than that of OF3. Such a relationship between esterification degree and Mw had been already reported for flax pectins (*43*). It is possible that during fruit development nonmethylesterified pectins had been partly depolymerized due to polygalacturonase activity.

Two acidic fractions of BHCl, namely, HF2 and HF3, were also enriched in galacturonic acid and might consist of residual homogalacturonans not fully extracted with chelating agents. Altogether, the homogalacturonans (in OF2 + OF3 + OF5 + HF2 + HF3) accounted for about 28 ± 3% of the total solubilized pectic components and 13 ± 2% of BAIR.

The fraction HF1, which was not retained on the column, was the major component of BHCl fraction. Its SEC profile was very similar to that of the bulk fraction BHCl (not shown). This pool HF1 accounted for 76 ± 4% of the total sugars recovered from the anion exchanger. It contained mostly galactose (>96%) together with a small amount of arabinose and glucose. Its sugar composition was very similar to AF1 and AF2 ones, which together accounted for about 60% of BOH fraction. Methylation analysis (**Table 4**) gives the major structural features of the BHCl polysaccharides. It largely consists of (1→4)-linked galactosyl (2,3,6-Gal represented 82% of the identified peaks), together with (1→2)-linked and (1→2,4)-linked rhamnosyl (3,4-Rha percentage being about 1% and 3-Rha about 2%, respectively), and a few (1→5)-linked arabinosyl (2,3-Ara accounting for about 1%) residues. (1→4)-Linked glucosyl and (1→4)-linked xylosyl residues were also present at low levels (2,3,6-Glc and 2,3-Xyl being detected at 1 and 1%, respectively). More information about the structure of the galactan present in the BHCl fraction was obtained by incubating it with endo-β-(1→4)-D-galactanase. The elution patterns on SEC of the galactan as well as its digest (recovered in the total volume) showed that this enzyme cleaved the backbone and converted the galactan into fragments (not shown). HPAE-PAD chromatographic analysis of the generated fragments indicates the presence of galactose and two other oligomers (not shown). On the basis of these observations, it may be concluded that the purified galactan was β-(1→4)-linked. HF5 also had a high content of galactose (>75% of the total NSs). Despite its late elution, its level in galacturonic acid was low, suggesting interaction types other than ionic ones with the DEAE-cellulose gel. Linkage analyses would be interesting to know whether they are 1→4 or 1→3, 1→6 or 1→3,6. Altogether (from HF1 + AF1 + AF2), the β-(1→4)-galactans accounted for 41 ± 3% of the extracted pectic fractions and for 19 ± 2% of BAIR.

As already noticed above, the two major fractions of BOH, namely, AF1 and AF2, consisted mainly of galactans. The difference of elution between AF1 and AF2 was due to the level

Table 3. Yields and Sugar Composition of Fractions Recovered from DEAE-Cellulose Chromatography of BOX, BHCl, and BOH Extracted Polymers

	yield NS[a]	yield GalA[b]	Rha[c]	Fuc[c]	Ara[c]	Xyl[c]	Man[c]	Gal[c]	Glc[c]	GalA[c]	GalA/Rha	(Gal + Ara)/Rha
BOX	100	100	1.5	tr	4.8	2.1	0.6	33.0	2.4	55.5	37.0	25.2
OF1	44.0	1.7	nd	nd	7.1	12.0	5.7	24.6	44.8	5.6	nd	nd
OF2	17.6	36.1	tr	tr	6.0	0.7	0.4	13.8	3.2	75.9	nd	nd
OF3	15.3	46.0	1.5	nd	3.5	0.2	nd	11.8	0.9	82.2	54.8	10.2
OF4	nd	nd	tr	tr	100					nd	nd	nd
OF5	23.1	16.2			7.7	10.1	2.8	14.2	13.7	51.4	nd	nd
BHCl	100	100	1.4	tr	1.2	0.1	tr	75.5	0.9	20.6	14.7	54.8
HF1	76.4	21.1		0.1	1.6			96.2	0.7	1.3	nd	nd
HF2	8.3	6.0	tr		13.5			10.0	12.6	63.9	nd	nd
HF3	2.8	16.2	0.5	tr	9.9		1.2	7.5	7.0	73.9	147.8	167.6
HF4	1.4	10.4		tr	100					nd	nd	nd
HF5	11.1	46.2	1.7	tr	10.2	2.3		76.8	5.5	3.5	2.1	51.2
BOH	100	100	1.1	tr	5.6	1.1	0.8	69.2	5.7	16.5	15	68
AF1	39.6	4.5		0.4	4.0	0.3	1.3	83.0	9.6	2.0	22	nd
AF2	22.2	15.4	0.3	0.3	8.3	0.5	1.3	79.4	3.2	6.6	22	nd
AF3	13.8	42.6	1.1	tr	12.0	0.4	1.0	58.9	2.1	24.4	22.2	nd
AF4	7.9	19.6		tr	22.1	0.8		51.2	2.7	23.2	nd	nd
AF5	16.4	17.8	0.8	tr	10.5	11.0	21.9	25.1	12.7	18.0	22.5	nd

[a] Weight percentage of total NS recovered. [b] Weight percentage of total UA recovered. [c] Percentage mol; nd, not determined; tr, trace; –, not detected. Except when otherwise stated, each value was the mean of at least two replicates and the standard deviation was in the range of 5% relative to the mean.

Table 4. Methylation Analysis of the BHCl Fraction Obtained from *B. hispida* Fruit

methylation product[a]	*m/z* values	peak area[b]
2,3,4,6-Gal	43, 45, 87, 102, 118, 129, 145, 161, 162, and 205	1
2,3,6-Gal	43, 45, 102, 113, 118, 129, 162, and 233	82
2,3,4-Gal	43, 87, 102, 118, 129, 162, 189, and 233	2
2,4,6-Gal	43, 45, 101, 118, 129, 161, 234, and 277	2
2,6-Gal	43, 45, 87, 118, 129, and 305	2
2,4-Gal	43, 87, 118, 189, 234, and 305	tr
2,3-Ara	43, 102, 118, 129, 162, 189, and 233	1
3,4-Rha	43, 89, 115, 131, 175, 190, and 234	1
3-Rha	43, 88, 101, 130, 143, 190, and 203	2
2,3-Xyl	43, 118, 162, 189, and 233	1
2,3,6-Glc	43, 45, 102, 113, 118, 129, 162, and 233	1
2,3-Glc	43, 102, 118, 127, 162, 201, 261, and 305	tr

[a] 2,3,4,6-Gal denotes 1-O-acetyl-2,3,4,6-tetra-O-methylgalactitol, etc. [b] Percentage of total area of the identified peaks. tr, trace. Each peak area value, which is given to its nearest whole number, was the mean of two replicates and the standard deviation was in the range of 5% relative to the mean.

of galacturonic acid. Despite the alkali treatment, but with almost no GalU, AF1 was not retained on the gel. On the other hand, AF3 and AF4 were very similar and contained about 25% galacturonic acid, 50−60% galactose, and 12−22% arabinose. The high amount of mannose present in the AF5 fraction probably arose from galacto- and/or glucomannan present in the mesocarp. Their late elution could result from two mechanisms: (i) being solubilized with KOH their UAs might have been saponified and (ii) hydrophobic and/or hydrogen bonds with the cellulose network of the anion exchanger might occur.

Apart from homoglacturonan and galactan, the presence of acidic arabinan, able to bind to the anion exchanger, was detected in OF4 and HF4 where arabinose almost amounted to ~100% of the total NSs. Because of the low percentage of these fractions, the level of UA could not be estimated.

In conclusion, this study has shown that about half of *B. hispida* fruit alcohol insoluble residues consisted of pectins. Two main populations of pectin, β-(1→4)-linked-D-galactans and homogalacturonans, were extracted, almost specifically using successive extractants. Their average physicochemical behavior was discussed as a function of their composition. Nonbranched and partially methylesterified homogalacturonans gave a more viscous solution than galactans, despite the higher Mw of the latter. The dilute alkali-extracted pectic polysaccharides, which had been remodeled due to acid and alkali treatments, displayed the highest viscosity. The high viscosity might be due to the complex acidic polysaccharides in addition to galactans. Some interactions between them could increase viscosity of the mixture. Further research will be directed toward a more detailed characterization of the purified polysaccharides in order to obtain information about the gelling ability of the isolated pectic polysaccharides, as it depends, inter alia, on solubility and viscosity (*19*).

ABBREVIATIONS USED

BAIR, alcohol insoluble residues of *Benincasa* fruit; BOX, BHCl, and BOH, ammonium oxalate, dilute HCl, and cold dilute alkali extracts from BAIR, respectively; DA, degree of acetylation; DM, degree of methylesterification; FT-IR, Fourier transform infrared; GLC/MS, gas liquid chromatography/mass spectrometry; Mw, molecular weight; NS, neutral sugar; UA, uronic acid; SEC, size exclusion chromatography.

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