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Structural Characterization of Oligosaccharides and Polysaccharides from Apple Juices Produced by Enzymatic Pomace Liquefaction

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Eight apple pomace liquefaction juices were produced to characterize soluble cell wall material released by the action of pectolytic and cellulolytic enzyme preparations. Very high colloid values from 9.7 to 19.6 g/L were recovered from the juices by ethanol precipitation. The crude polysaccharides consisted mainly of galacturonic acid (49−64 mol %), arabinose (14−23 mol %), galactose (6−15 mol %), and minor amounts of rhamnose, xylose, and glucose. Separation of the polysaccharides by anion-exchange chromatography yielded one neutral, one slightly acidic, and one acidic polymer accounting for 60% of total colloids. Preparative size exclusion chromatography of the acidic fractions resulted in four polymers of different molecular weights and different sugar compositions. Among them, high molecular weight arabinans and rhamnogalacturonans as well as oligomeric fractions consisting of only galacturonic acid could be found. Linkage studies were performed on neutral fractions from anion-exchange chromatography and size exclusion chromatography. They revealed highly branched arabinans, xyloglucans, and mainly type I arabinogalactans.

KEYWORDS: Apple; oligosaccharides; polysaccharides; pomace liquefaction; pectinases; cellulases; per-O-methylation

INTRODUCTION

For the past 20 years (*1*), pectolytic enzyme treatment of apple mash has been part of the production of clear apple juice concentrates. Enzymatic treatment of mash reduces the water binding capacity of the cell wall polysaccharides, thus leading to lower viscosities and an easier dejuicing. A newer technique is the enzymatic liquefaction process. There, the apple mash is treated with pectinases and cellulases at 50 °C to reach a more complete breakdown of cell walls (*2*, *3*). This process is also known as total liquefaction (*4*, *5*). The objective of both techniques is a higher juice yield, even if storage apples are processed. Despite high juice yields, the total liquefaction process has some major disadvantages such as poor sensory qualities and an increased tendency to browning reactions (*36*, *37*). Additionally, the total liquefaction is restricted because of high enzyme costs and legal regulations as cellulases are not mentioned in the European Union (EU) guideline for fruit juices.

The technological disadvantages can be overcome by a two-step operation. In the first step, an usual mash processing with pectinases is used. The resulting A-juice or premium juice can be processed separately. In the second step, the pomace is mixed with hot water, and liquefaction enzymes are added. After a certain incubation time, the pomace is extracted once more. Pomace liquefaction with pectinases and cellulases is already a common technique in some countries of eastern Europe and North and South America.

The use of cellulase-containing enzyme preparations to liquefy apple pomace was investigated in this study. Due to the specific enzymatic treatment at higher temperatures in the liquefaction step, the apple cell walls are strongly affected. The analytical characterization of the corresponding extraction juices has already been reported (*6*). As calculated to an equal juice strength of 12 °Brix, there was an increase in complex carbohydrates, total acidity, minerals, and polyphenols. The most distinct alterations were the high amount of D-galacturonic acid, oligosaccharides, and polysaccharides transferring into the extraction juice. Galacturonic acid released during the enzymatic treatment contributed essentially to the total titrable acid of the extraction juices.

Usual apple mash treatment with pectinases already leads to increasing colloid concentrations as compared to nontreated control samples (*7*). A subsequent treatment of the pomace with high dosages of liquefying enzymes will even exceed these

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Table 1. Details of Juice Production

premium juice A	mash treatment[a] (dosage)	juice separation	extraction juice B	pomace treatment[a] (dosage)	juice separation
1A	Pectinex Smash[p] (80 ppm) (Novozymes, Switzerland)	press	1B	water only (control)	press
2A	Rapidase[p] (100 ppm) (DSM, France)	press	2B	Rapidase Pomalique[c,p] (250 ppm) (DSM, France)	press
3A	Pectinex Smash (80 ppm) (Novozymes, Switzerland)	press	3B	Pectinex AFP-L 2[p] (250 ppm) (Novozymes, Switzerland)	press
4A	Rohapect MA plus[p] (100 ppm) (Roehm, Germany)	press	4B	Rohapect AP1[p] (200 ppm) Rohalasec (100 ppm) (Roehm, Germany)	press
5A	Rohapect MA plus (100 ppm) (Roehm, Germany)	press	5B	Rohapect AP1 (200 ppm) Rohament CL[c] (200 ppm) (Roehm, Germany)	decanter
6A	Rohapect MA plus (100 ppm) (Roehm, Germany)	decanter	6B	Rohapect AP1 (200 ppm) Rohament CL (200 ppm) (Roehm, Germany)	press
7A	Pectinex Smash (80 ppm) (Novozymes, Switzerland)	press	7B	Pectinex AFP-L 2 (250 ppm) (Novozymes, Switzerland)	decanter
8A	Pectinex Smash (80 ppm) (Novozymes, Switzerland)	press	8B	Ultrazym AFP-L[c,p] (250 ppm) (Novozymes, Switzerland)	decanter

[a] Main activities: [c], cellulases; [p], pectinases.

values. With regard to juice technology, high colloid concentrations cause low flux rates during ultrafiltration (*33*). Additionally, high dosages of fining agents such as gelatin or silica sol will be necessary for a sufficient clarification. Besides technological aspects, pectic polysaccharides have also positive properties, particularly as dietary fibers in human nutrition (*32*).

There is a lot of data available concerning the composition of colloids from apple juices that were produced in the traditional way (*8*−*12*) or by total liquefaction (*2*, *13*). Oligosaccharides or polysaccharides resulting from enzymatic pomace liquefaction have not been investigated yet. In the European Union, the pomace liquefaction process is not allowed. Apart from legal aspects, there is also no nutritional assessment of the corresponding juices. The present work focuses on the analytical composition of soluble cell wall colloids of apple extraction juices produced by enzymatic pomace liquefaction.

MATERIALS AND METHODS

Juice Production, Sampling. Juices 1−4 were produced in 1998 from cider apples. Samples 5−8 were manufactured in 1999 from cv. Jonagold. Every batch was done with 650−800 kg. After washing, the apples were crushed in a Rätz mill (Kleemann, no. 1−4) or a hammer mill (Bellmer BAC, no. 5−8) followed by mash treatment with different pectolytic enzyme preparations over 1 h at 20 °C.

A-Juices. Mashes were dejuiced with a horizontal press (Bucher HP-L 200) or a decanter (Flottweg Z23-3), separated (Westfalia SAR 3036), filled into glass bottles, and pasteurized for 25 min at 85 °C.

B-Juices, Pomace Liquefaction. Pomace obtained from the press or the decanter was resuspended in hot demineralized water (1:1, w/v, 95 °C) and treated with different enzyme preparations over 2 h at 50 °C in a temperature-controlled stirring tank. Pomace was extracted again with a press or a decanter, and the resulting juices were treated as above. Details of the trials are presented in **Table 1**.

Isolation of Colloids. Juice polysaccharides were isolated by alcoholic precipitation. Juices (300 mL) were centrifuged (3000*g*), and 5 volumes of ethanol (96%) was added. The mixture was thoroughly stirred for 5 min and stored overnight in the cold. After centrifugation, the supernatant was discarded, and the residue was dissolved in 100 mL of water (60 °C). Water insoluble material was removed by centrifugation. The supernatant was precipitated a second time with 5 volumes of ethanol. The resulting residue obtained after centrifugation was freeze-dried, further dried in a vacuum oven, and finally weighed.

Anion-Exchange and Analytical Size Exclusion Chromatography. Juice colloids were separated by column chromatography on a weak anion-exchange material (DEAE-Sepharose CL-6B, Pharmacia) using a step gradient consisting of bidistilled water and 50 and 250 mM NaCl. The molecular weight distributions of polymer fractions were determined by gel filtration on a Pharmacia FPLC system equipped with a Superose 12 column and a refractive index detector. Both methods were described in detail elsewhere (*12*, *14*).

Preparative Fractionation of Purified Colloids on a Superose 6 Preparative Grade Column. Acidic fractions (250 mM NaCl) from anion-exchange chromatography were further separated by size exclusion chromatography on a 50 × 1.6 cm column (Pharmacia HR 16/50), using 0.2 M NaCl as eluent at a flow rate of 30 mL/h and refractive index detection. Sampling of 2 mL of dissolved material (18 mg/mL) and peak collection were controlled by a Pharmacia FPLC system equipped with a P-500 pump. Fractions were desalted by column chromatography on Sephadex G-25 material (18 × 5 cm, Pharmacia) and freeze-dried.

Sugar Composition of Pectins. Neutral and acidic sugars were determined after Saeman hydrolysis (*15*) of the polysaccharides followed by HPAEC on a Dionex Bio-LC system. In prehydrolysis, exactly 10−15 mg of dried material was weighed into glass vials and 125 μL of sulfuric acid (72%, w/w) was added. Samples were sonicated for 45 min at ambient temperature. For the main hydrolysis, 1.35 mL of water was added, and the vials were placed in a heating block for 60 min at 120 °C. After cooling to room temperature, the vials were transferred completely into 50 mL flasks and made up to volume with bidistilled water. To save time in larger test series, neutral sugars and D-galacturonic acid were determined in separate runs. Filtered (0.2 μm) samples (20 μL) were injected onto a 4 × 250 mm Carbo Pac PA-1 column, guarded with a 4 × 50 mm Carbo Pac PA-1 precolumn, both at 15 °C in a Jasco column thermostat. Elution of neutral sugars was performed during 0−24 min with 12 mM NaOH, followed by flushing from 24.1 to 34 min with 400 mM NaOH and then equilibration to 12 mM NaOH. For D-galacturonic acid, the eluent was 400 mM NaOH (Baker). In both cases, the flow rate was 1.0 mL/min and the detection was electrochemical with pulsed amperometry. Quantitation was carried out using peak areas from external calibration with standard solutions (Merck). Analysis was done in duplicate.

Linkage Study by Permethylation. Methylation analysis was performed with butyllithium and methyl iodide in dimethyl sulfoxide (*17*). The per-O-methylated polysaccharides were hydrolyzed with 2 M trifluoroacetic acid and transformed to their alditol acetates according to the method of Harris et al. (*34*). The partially methylated alditol acetates (PMAAs) were separated by GLC on a fused-silica capillary column (SP 2340, 25 m × 0.32 mm, Supelco) after split injection and FID detection (Carlo Erba Mega GC, HP ChemStation software). The resulting peak areas were corrected by their effective carbon response factors according to the method of Sweet et al. (*18*), originally based on the theoretical ionization potential of organic analytes in a flame ionization detector. The PMAA peaks were identified by comparison of mass spectra from GC-MS spectrometry (5890/2 gas chromatograph, MS-engine, Hewlett-Packard) using the same column and similar chromatographic conditions.

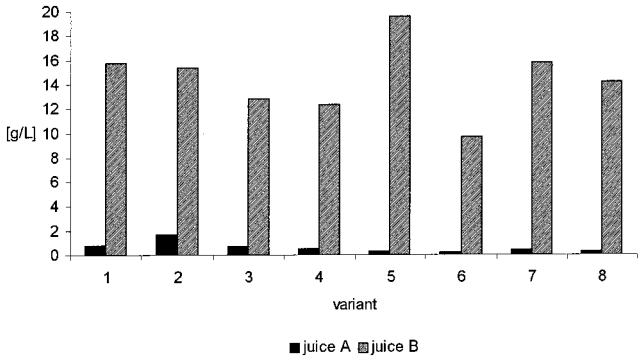

Figure 1. Colloid concentrations expressed as grams per liter, referring to 12.0 °Brix juice strength of the premium juices (A) and the corresponding pomace liquefaction juices (B).

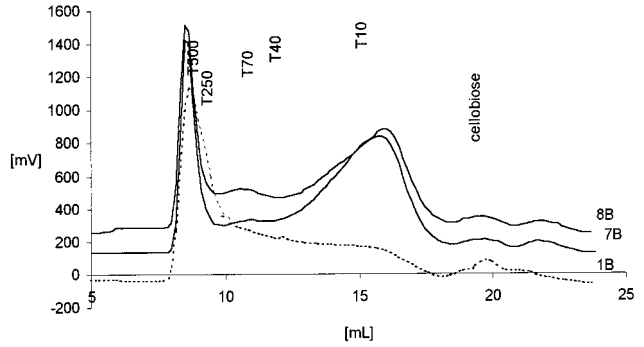

Figure 2. Molecular weight distribution of the raw colloids from samples 1B (control), 7B, and 8B. (Markers: T10–T500 dextran standards, Pharmacia; and cellobiose, Merck, disaccharide standard.)

RESULTS

The isolated raw colloids were at first separated by anion-exchange chromatography on DEAE-Sepharose CL 6B, yielding three different fractions named H_2O, 50 mM NaCl, and 250 mM NaCl. The neutral fraction H_2O and the weakly acidic 50 mM NaCl represented only 20 and 5%; the fraction 250 mM was an acidic polymer accounting for 60% of total colloids. Although there were certain losses of material due to interactions between sample and column material, desalting, and freeze-drying respectively, no specific fraction was lost in this process. Fraction 250 mM was further separated by size exclusion chromatography on Superose 6 preparative grade.

Colloid Concentrations. The colloid concentrations of the different juices are shown in **Figure 1**. Relatively high values of 9.7−19.6 g/L were found for the extraction juices. Calculated to a juice strength of 12 °Brix, the colloid concentrations increased by factors of 10−65 from juices A to B.

Molecular Weight Distributions. Figure 2 shows selected and representative molecular weight distributions of pectins obtained from the non-enzyme-treated control 1B, the pectinase-treated sample 7B, and the cellulase-treated sample 8B. High molecular weight polysaccharides were present in each of the samples. They eluted in the void volume of the column and indicated molecular weights of ≥280 kDa. The control sample consisted mainly of these high molecular weight pectins. Its molecular weight distribution showed only a few fragments eluting in a broad range from 10 to 70 kDa. As a result of enzymatic degradation, the enzyme-treated pectins 7B and 8B additionally showed polysaccharides having apparent molecular weights between 1 and 40 kDa. Small peaks occurred at 70 kDa and at a molecular mass similar to that of cellobiose, which was used as disaccharide standard for the column calibration.

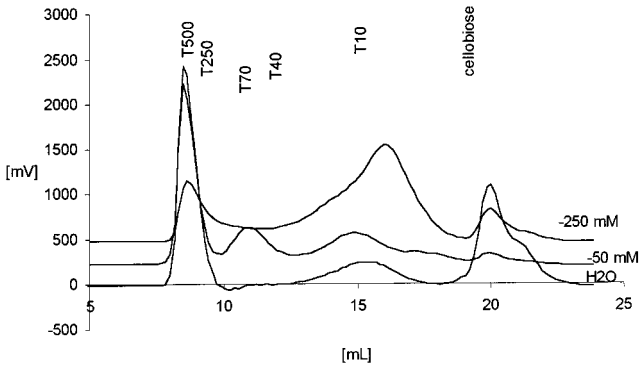

Figure 3. Molecular weight distribution of DEAE fractions H_2O and 50 and 250 mM NaCl from juice 8B.

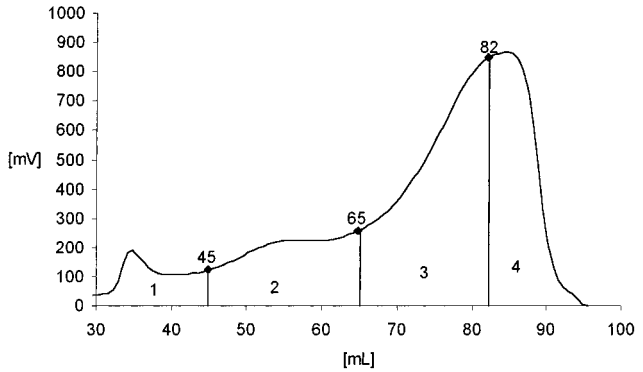

Figure 4. Preparative fractionation of acidic pectins (DEAE 250 mM, juice 8B) on Superose 6 preparative grade column, fractions 1–4.

Enzymatic treatment of pomace resulted in large amounts of small pectin fragments in the low molecular weight range of <10 kDa.

The molecular weight distributions of the DEAE fractions from juice 8B are shown in **Figure 3**. The elution profiles of the H_2O and 50 mM NaCl fractions were similar except for a peak in the molecular range near 70 kDa in the latter fraction. The main peaks eluted in the void volume of the Superose 12 column representing high molecular wieght polysaccharides of >280 kDa. The H_2O fraction contained substances with molecular masses of disaccharides. The acidic fraction 250 mM showed higher molecular weights and a more complex distribution with polymers eluting in a broad range of <10 and up to 40 kDa. The high molecular weight peak in the void volume appeared to be less intense than the corresponding peaks of H_2O and 50 mM fractions. Unlike the latter, DEAE 250 mM is a heterogeneous mixture of different polysaccharides that were depolymerized during enzyme treatment of pomace.

Separation of Fraction 250 mM by Size Exclusion Chromatography. Size exclusion chromatography of fraction 250 mM from juice 8B on Superose 6 preparative grade revealed four fractions as indicated in **Figure 4**. Five sample runs were done to obtain sufficient material for further characterization.

The molecular weight distributions (Superose 12, Pharmacia) of the collected fractions 1−4 are shown in **Figure 5**. Four different populations could be isolated by size exclusion chromatography on the Superose 6 preparative grade column. Fraction 1 consisted of a sharp peak eluting in the void volume of the Superose 12 column. These high molecular weight polymers were composed of neutral sugars only, accounting for 68 mol % arabinose, 12 mol % galactose, 11 mol % xylose, and 10 mol % rhamnose (for detailed data see **Table 2**). Fraction 2 was eluted as one main peak in the void volume and a shoulder

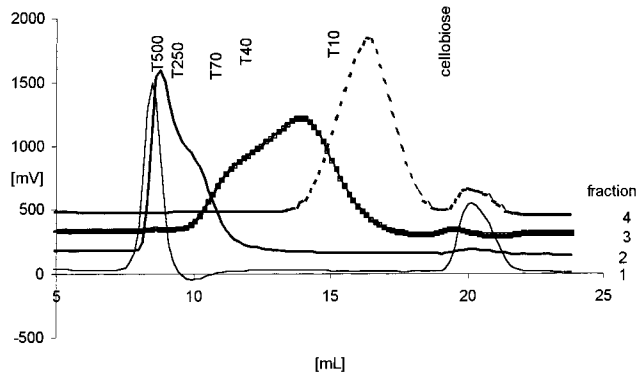

Figure 5. Molecular weight distribution of fractions 1–4 obtained from DEAE fraction 250 mM NaCl of juice 8B by preparative size exclusion chromatography on Superose 6 preparative grade.

Table 2. Sugar Composition (Mole Percent) and Total Sugar Content (Percent Weight) of Fractions 1–4 Obtained by Preparative Size Exclusion Chromatography on Superose 6 Preparative Grade of DEAE Fraction 250 mM NaCl of Juice 8B

fraction	fucose	rhamnose	arabinose	galactose	glucose	xylose	galA	total sugar
1	nd[a]	10	68	12	nd	11	nd	36
2	nd	3	30	5	nd	2	59	63
3	3	3	3	2	nd	nd	90	63
4	nd	nd	0.4	nd	nd	nd	99	60

[a] nd, not detected.

Table 3. Sugar Compositions (Mole Percent) and Total Sugar Content (Percent Weight) of the Raw Colloids Isolated from the Produced Juices

sample	rhamnose	arabinose	galactose	glucose	xylose	galA	total sugar
1A	2	14	10	5	<1	68	29
1B	3	20	8	11	1	57	36
2A	2	8	7	14	3	66	28
2B	4	23	8	15	2	49	35
3A	2	13	6	6	<1	72	29
3B	4	17	7	6	2	64	30
4A	3	15	11	3	1	66	31
4B	5	23	6	7	1	57	35
5A	2	12	12	3	2	69	60
5B	4	20	9	7	2	58	63
6A	2	12	14	3	2	68	56
6B	5	19	10	2	3	60	52
7A	2	10	11	nd[a]	nd	77	61
7B	3	14	15	nd	3	64	71
8A	2	12	12	nd	nd	74	57
8B	3	16	13	2	10	56	83

[a] nd, not detected.

with apparent molecular masses ranging from 70 to 200 kDa. This fraction consisted mainly of galacturonic acid (59 mol %) and arabinose (30 mol %). Galactose, rhamnose, and xylose also occurred in lower amounts. Fraction 3 eluted in a broad peak with molecular masses of 10−70 kDa. Galacturonic acid increased considerably accounting for 90 mol %. Neutral sugars were identified as arabinose, galactose, fucose, and rhamnose in equal amounts. Low molecular weight (<10 kDa) pectins were present in fraction 4 and consisted almost completely of galacturonic acid.

Sugar Composition. Table 3 summarizes the monomer composition of raw colloids isolated from juices A and B. Galacturonic acid dominated the composition of all samples.

Table 4. Sugar Composition (Mole Percent) and Total Sugar Content (Percent Weight) of the DEAE Fractions

sample	fraction	rhamnose	arabinose	galactose	glucose	xylose	galA	total sugar
1B	H_2O	nd[a]	17	33	49	1	nd	64
	50 mM	3	44	15	29	1	8	90
	250 mM	6	36	6	1	1	50	71
2B	H_2O	1	12	25	58	2	nd	51
	50 mM	2	25	16	55	2	nd	63
	250 mM	3	24	5	nd	2	66	56
3B	H_2O	nd	22	39	37	nd	nd	36
	50 mM	2	58	18	20	2	nd	81
	250 mM	2	14	4	2	2	76	70
4B	H_2O	3	30	26	24	3	14	27
	50 mM	2	36	15	14	3	30	81
	250 mM	2	15	6	nd	nd	77	66
5B	H_2O	4	35	23	24	6	7	27
	50 mM	4	34	21	22	4	15	55
	250 mM	2	11	3	nd	<1	83	55
6B	H_2O	5	35	19	17	7	14	55
	50 mM	3	32	25	20	7	12	78
	250 mM	3	11	3	nd	1	82	59
7B	H_2O	5	31	19	13	10	22	38
	50 mM	4	45	22	11	8	10	80
	250 mM	3	12	3	nd	3	79	55
8B	H_2O	5	44	28	9	13	nd	28
	50 mM	2	51	20	8	6	13	66
	250 mM	3	18	4	nd	2	72	62

[a] nd, not detected.

The main neutral sugars of the premium juices were arabinose and galactose. The contents of glucose strongly varied from not detectable in samples 7A, 7B, and 8A to 15 mol % in sample 2B. Rhamnose concentrations slightly increased in the extraction juices as compared to premium juices. A relatively high xylose content of 10 mol % was found in sample 8B. Fucose occurred only in traces (data not shown).

In all cases the total yield of the sugars was <100%, which was due to hydrolysis problems, reversion reactions of sugars, and impurities of the polysaccharides, particularly phenolic substances and protein moieties (*35*). As a consequence, the total sugar contents of the pectins from juices 5−8 are substantially higher than those from juices 1−4. Samples 5−8 were produced from cv. Jonagold, a table apple with low polyphenol concentrations. Samples 1−4 were prepared from cider apples with high amounts of polyphenols (*6*).

Further separation of the colloids by anion-exchange chromatography resulted in a neutral fraction, a weakly acidic fraction, and one acidic fraction. The more neutral polymers consisted mainly of arabinose (12−44 mol % in H_2O fractions and 25−58 mol % in 50 mM NaCl fractions), galactose (19−39 and 15−25 mol %, respectively), and glucose (9−58 and 8−55 mol %, respectively). The detailed monosaccharide composition is given in **Table 4**. Further sugars found were rhamnose, varying from not detectable to 5 mol %, and xylose, varying from not detectable to 13 mol % in fraction H_2O of sample 8B. Galacturonic acid also occurred in these polysaccharides. This was due to high concentrations of galacturonosyl polymers, which were not completely bound to the Sepharose CL-6B resin and coeluted with the neutral polysaccharides. At an ionic strength of 250 mM pectin fragments with 50−83 mol % of galacturonic acid could be eluted from the anion-exchange column.

With regard to the monomer composition of the different colloids, arabinose and galactose dominated in varying ratios.

Table 5. Methylation Analysis (Mole Percent) of DEAE Fractions H_2O and 50 mM

	DEAE H_2O								DEAE 50 mM							
linkage type	1B	2B	3B	4B	5B	6B	7B	8B	1B	2B	3B	4B	5B	6B	7B	8B
1,2-linked rha*p*					1.2	1.2	1.3	1.0						1.1	0.9	1.0
1,2,4-branched rha*p*						1.2	2.2	1.7								
terminal ara*f*	9.0	9.9	5.1	13.1	18.6	16.1	15.1	12.3	15.6	9.7	14.6	3.5	10.5	16.4	26.4	26.2
terminal ara*p*					1.2	1.4				1.0	1.1	3.0		2.8	2.1	2.2
1,2-linked ara*f*	1.3	1.5		1.5		2.1	1.4	2.4								
1,3-linked ara*f*	1.4	1.4	1.5	1.7	1.8	1.7	2.6	2.3	2.1	2.6	3.1	8.6	1.7	4.5	4.7	5.1
1,5-linked ara*f*	13.3	9.3	16.7	13.3	20.4	17.3	23.8	20.2	10.6	9.5	14.5	14.8	7.2	11.0	18.0	20.4
1,3,5-branched ara*f*	8.9	8.2	7.1	13.3	13.0	12.6	14.9	15.1	5.9	9.2	13.1	11.5	5.3	7.3	9.3	12.8
1,2,5-branched ara*f*		1.4	1.1	1.6	1.5	1.4	1.7	1.5	1.1	1.9	2.7	1.9			1.7	1.8
terminal xyl*p*	1.9	1.2	2.5	5.8	5.4	7.0	8.7	5.6	1.2	1.4	2.3	2.4	1.4	3.4	6.0	2.5
1,2-linked xyl*p*	7.1			1.6	4.9	3.6	2.6	2.9	4.6							2.4
terminal gal*p*		1.7	-	1.0			1.7	1.2				1.0			1.8	
1,3-linked gal*p*				1.1		1.3	2.3	2.4	2.1	2.4	2.8	1.1	3.1	5.5	4.6	3.8
1,4-linked gal*p*	11.0	12.9	24.4	13.8	9.7	9.0	7.9	11.0	1.4	6.8	2.5	19.2	1.5	2.8	1.7	1.6
1,6-linked gal*p*							1.0	0.8		1.1				1.9	2.0	1.1
1,3,4-branched gal*p*	1.9	2.9	1.1	1.7					1.2	1.2	1.3	1.4	1.0			
1,2,4-branched gal*p*	1.2	1.5	1.1	1.0												
1,4,6-branched gal*p*	1.1	1.2	1.8	1.9				1.0								
1,3,6-branched gal*p*						1.1	2.5	3.4	5.0	10.0	9.6	1.5	7.9	17.4	12.6	9.8
terminal glc*p*	3.2	7.7	4.5	2.6	1.6	1.3	2.1	1.9	3.5	7.1	1.7	3.8	2.0	1.5		
terminal glc*p*	2.1	6.6	2.4	1.4	1.2	1.5			2.2	3.7	1.8	1.8	2.7	1.4		
1,4-linked glc*p*	25.4	18.7	19.7	12.8	9.0	6.9	1.9	5.0	36.3	18.1	14.8	16.4	50.2	13.3	2.4	2.9
1,4,6-linked glc*p*	2.4	5.8	1.7	3.6	2.5	4.1		1.6	2.9	6.9	1.9	1.4	3.3	3.9		
?	3.5	5.9	5.8		3.5	3.0	1.7	1.8								

The juices were starch-free, but especially in the H_2O and 50 mM polymers of samples 1B and 2B glucose occurred at 58 and 55%, respectively. Xylose contents increased in the DEAE fraction H_2O, accounting for 6−13% in the samples 5−8B, indicating xyloglucans or xylogalacturonans (*38*) released from the apple cell wall. Fraction 250 mM isolated from the enzyme-treated juices had the lowest yield of neutral sugars. It accounted for an average of 25%, whereas the non-enzyme-treated control contained 50% neutral sugars. Together with high values for arabinose, the contents of galacturonic acid and rhamnose showed that these pectic fragments were derived from the rhamnogalacturonan zones of the pectic molecule.

Linkage Study of Neutral Polysaccharides. Samples H_2O and 50 mM resulting from DEAE separation were per-O-methylated. **Table 5** shows the distribution of the corresponding methyl ethers (>0.5 mol %), which differed from sample to sample. The H_2O fractions consisted of a complex mixture of polysaccharides. Their α-1,5-linked arabinosyl units as backbone were branched in positions 2 and 3 with arabinosyl residues and dominated in all samples in varying ratios. Terminal and 1,3,5-branched arabinose predominated, indicating highly branched arabinans. *β*-1,4-Linked glucose main chains with terminal and α-1,2-linked xylosyl residues were found in differing concentrations, pointing to xyloglucans, which were released from apple cell walls during pomace extraction. The colloids of the non-enzyme-treated control 1B consisted also of a high proportion of *β*-1,4-linked glucosyl residues and α-1,2-linked xylosyl side chains, respectively. Different methyl ethers of galactose could be identified. Galactose was mainly in the 1,4-linked position with several side chains at positions 2, 3, and 6 representing type I arabinogalactans. Only traces of type II arabinogalactans were detected, which were composed of a 1,3-linked galactosyl backbone carrying 1,6-linked galactosyl side chains. Similar to the H_2O samples, fraction 50 mM consisted mainly of these three different neutral polymers except for samples 7B and 8B, in which 1,4-linked glucans dominated. Branched arabinans also occurred, probably as side chains from type I and type II arabinogalactans. Slightly higher amounts of 1,3- and 1,6-linked galactans (type II) were present in this fraction.

Table 6. Methylation Analysis (Mole Percent) of Fractions 1 and 2 Obtained from Preparative Size Exclusion Chromatography of DEAE Fraction 250 mM NaCl of Juice 8B on Superose 6 Preparative Grade

linkage type	fraction 1	fraction 2
1,2-linked rha*p*	1.9	2.3
1,2,4-branched rha*p*	1.6	1.2
terminal ara*f*	29.0	28.1
1,2-linked ara*f*		1.4
1,3-linked ara*f*	3.0	3.1
1,5-linked ara*f*	23.2	25.0
1,3,5-branched ara*f*	13.4	14.1
1,2,5-branched ara*f*	3.2	3.8
terminal xyl*p*	3.7	
1,2-linked xyl*p*	1.9	3.8
1,3-linked gal*p*	1.1	1.8
1,4-linked gal*p*	2.2	2.1
1,4-linked glc*p*	1.5	
?	14.3	13.3

Furthermore, fractions 1 and 2 obtained by preparative gel filtration on Superose 6 were per-O-methylated. These polysaccharides were predominantly composed of 1,5-linked arabinans with terminal arabinosyl residues. Minor amounts of galactose in the 3- and 4-linked positions occurred as well as rhamnose in the 2- and 2,4-linked positions (**Table 6**).

DISCUSSION

Extremely high colloid concentrations up to 20 g/L belong to the characteristic properties of juices from pomace liquefaction. The reason is the extensive depolymerization of the apple cell wall material during 2 h of enzymatic pomace treatment at optimum temperatures of 50 °C. The corresponding premium

juices contained usual colloid concentrations from 0.2 to 0.8 g/L except 2A, which had 1.7 g/L (*6*).

In general, the composition and structure of soluble pectins are similar to those isolated earlier from apples and apple juices (*2*, *9*, *12*, *13*, *20*−*24*). Neutral polysaccharides were predominantly composed of α-1,5-linked arabinans, type I arabinogalactan, and xyloglucan. Hemicelluloses were intensively released from the cell wall's matrix by stressing apple mash or pomace with enzymes. Endoglucanases released xyloglucans from the plant cell wall before digestion of cellulose fibrils. Xylose and glucose, which were only minor components of the raw colloids, could be strongly enriched in the neutral fractions by anion-exchange chromatography.

In plant cell walls α-1,5-linked arabinans occur as pectin side chains. Besides arabinose-rich pectins, Aspinall et al. (*25*) isolated a neutral arabinose-rich fraction consisting of α-1,5-linked arabinan chains highly branched with terminal arabinosyl residues or short arabinose side chains. Arabinans isolated from apple juices are involved in clarification and stabilization problems of apple juices and concentrates (*7*, *26*). Schols et al. (*11*) analyzed the composition of high molecular weight compounds in apple juices obtained by straight pressing, enzyme treatment of pulp, or liquefaction. The amount of colloid material in juice was strongly influenced by the processing method and enzyme preparation used. The high molecular weight material rich in arabinose and particularly present in liquefaction juices must be considered as a possible precursor of haze formation. Water extraction of the pomace without using enzymes also increases arabinan concentrations (*7*).

The main polysaccharides of premium juices from apples (*7*) and berries (*27*) are type II arabinogalactans. They consist of *β*-1,3-linked galactosyl main chains branched at position 6 with short *β*-1,6-galactan side chains and arabinosyl residues. In the juices from apple pomace liquefaction, only low amounts of type II arabinogalactans were identified. Probably, type II arabinogalactans aggregate and precipitate during pomace extraction because of the extensive enzymatic treatment, which removes arabinosyl side chains. Type II arabinogalactans are associated with hydroxyproline-rich proteins (*28*, *29*) of the cell wall and may be specified as arabinogalactan-proteins (AGP). Brillouet et al. (*30*) isolated an AGP from an apple juice. Enzymatic dearabinosylation of AGP with a purified α-L-arabinofuranosidase led to partial aggregation of residual galactan-proteins. The sugar composition of the rhamnogalacturonan polymers from DEAE fraction 250 mM differed from an analogous polymer isolated by Schols et al. (*2*) from apple juices processed by enzymatic liquefaction. There, higher arabinose contents and higher rha/galA ratios were found. In the course of these investigations no essential differences were established among the different enzyme preparations applied for pomace liquefaction. Concerning the analytical composition of pomace liquefaction juices (*6*) and the present data of the colloid composition, there are no technological and nutritional reasons against the use of cellulases in apple juice processing.

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