Effects of Commercial Pectolytic and Cellulolytic Enzyme Preparations on the Apple Cell Wall

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The action of three different commercial enzyme combinations on apple cell wall material has been examined in a model system under conditions of mash and pomace treatment by using an alcohol-insoluble substance prepared from apples. A part of the total dietary fiber, for example, galacturonan (pectin), appeared in the soluble fraction after enzymatic mash treatment. The soluble fraction increased intensely during pomace treatment. Furthermore, enzyme actions caused a change in the water-binding capacity of residues as well as changes in the monosaccharide composition and in the molecular weight distribution of saccharides in filtrates (soluble parts). The extent of decomposition of cell wall material and the increase of soluble oligomeric and/or polymeric dietary fiber components are caused by both the composition (pectinases, cellulases, and hemicellulases) and the activities of the enzyme preparations. The model experiments allow an insight into the reactions occurring during enzyme action on the plant cell wall, for example, during apple juice production using pectolytic and cellulolytic enzyme preparations.

Keywords: *Pectolytic enzymes; cellulolytic enzymes; cell wall decomposition; pectin; dietary fiber; alcohol-insoluble substance from apples*

INTRODUCTION

The cell wall of apples (*Malus domestica*) consists of different polysaccharides, especially pectin, hemicelluloses, and cellulose (*1*), as well as structural proteins and lignin. These polysaccharides influence processing (*2*), for instance, during production of juice products, and play a role as dietary fiber (*3*, *4*) in human nutrition.

In contrast to traditional methods, modern technology uses enzyme preparations for the production of apple juice (*5*, *6*). Important objectives for a pectinase treatment of apple mash are the improvement of pressability and throughput as well as the yield of juice (*7*). Besides pectolytic enzymes, cellulases were tested and applied in fruit juice technologies (*8*, *9*) during the past few years. At first, the concept of (total) liquefaction—which means the common application of pectolytic and cellulolytic enzyme preparations on the apple mash—was investigated extensively, but it was shown that a two-step technology consisting of a mash treatment with pectinases and, after pressing, a pomace treatment with pectinases and cellulases is advantageous with respect to yield, quality, and sensory properties (*10*, *11*).

By treatment with pectolytic, hemicellulolytic, and cellulolytic enzyme preparations, polysaccharides of the plant cell walls are partly depolymerized and solubilized to a different extent. This effect causes changes in the functional properties of fruit material (pressability, viscosity, water-binding properties, etc.) as well as in the yield and composition (including contents of polyphenols and dietary fiber) of apple juices. Therefore, alterations of cell walls or polysaccharides during such enzymatic treatments require investigation. Schols et al. (*12*) examined the influence of different manufacturing methods such as straight pressing, enzymatic pulp treatment, or liquefaction on the properties of juices obtained and on the amount and composition of polysaccharides. Higher amounts of polysaccharides were solubilized under conditions of liquefaction as a result of enzymatic action on the plant cell wall than with the other methods tested. Voragen et al. (*13*) reported the highest release of total saccharides from apple cell walls by using a combination of pectolytic enzymes and C_1 cellulase. Furthermore, the composition and molecular weight distribution of these solubilized fragments depend on the purified enzymes used.

Alcohol-insoluble substances (AIS) are a suitable model for the characterization of enzyme actions on fruits and vegetables for the production of juices, macerates, or related products. Such model experiments allow one to follow, step by step, the solubilization of individual polysaccharides from the cell wall and their further degradation in the liquid phase. It may be possible to optimize the enzyme composition and concentration from the results of such model experiments. Destruction of grown cell wall architecture and depolymerization of the polysaccharides are connected with changes in the functional and physicochemical properties of the plant material.

This study describes the action of commercial enzyme preparations during mash and pomace treatment on the AIS prepared from apples in a model system. The effect of three enzyme combinations on total dietary fiber (DF) and on galacturonan (pectin) in the soluble and insoluble fractions was analyzed. Furthermore, changes in the water-binding capacity (WBC) of residues as well as the monosaccharide composition and the molecular weight distribution of saccharide containing filtrates (soluble parts) were determined.

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MATERIALS AND METHODS

Preparation of AIS. Cv. Boskoop apples (weight = 109.9 ± 21.7 g) grown in the region of Potsdam, Germany, were harvested in the middle of October 1998. After a cold storage of 1 month, 10.3 kg of apples was used with skins and cores for the preparation of AIS. One part of cut fruits was crushed into small pieces in two parts of 96% EtOH using a blender and an Ultra-Turrax T25 (Jahnke & Kunkel, IKA Labortechnik) and boiled under reflux for 15 min. After separation of the liquid phase by suction and washing with hot 65% EtOH, the residue was extracted a second time. Then, the residue was washed sequentially with 65, 80, and 96% EtOH as well as with acetone. The vacuum-dried AIS was milled to a particle size of ≤0.5 mm.

Characterization of AIS. Total pectin was determined after alkaline de-esterification (1 h at 20 °C, pH 10.0), treatment with Rohament P (Röhm Enzyme, Darmstadt, Germany) (1 h at 20 °C, pH 4.5), and extraction with 0.5% aqueous EDTA (16 h at 20 °C, pH 6.0). Soluble pectin fractions were obtained by extraction with water or 0.5% EDTA (16 h at 20 °C, pH 6.0) (*14*). The content of galacturonan was determined according to the *m*-hydroxybiphenyl method (*15*). The degree of esterification was analyzed by titration of carboxyl groups before and after de-esterification (*16*). Total, soluble, and insoluble DF were measured by using the AOAC method (*17*). Contents of dry matter and ash were determined after heating of the AIS for 3 h at 105 °C and for 2 h at 525 °C, respectively.

Enzyme Preparations and Dosages. Enzyme preparations used for mash treatment were Pectinex Smash (Novo Nordisk Ferment, Neumatt, Switzerland) and Rapidase HP (Gist-Brocades, France) with standardized pectolytic and hemicellulolytic activities according to the manufacturers. Rohapect MA Plus (Röhm) had standardized pectolytic activity only. For pomace treatment, Rapidase Pomaliq Extra (Gist-Brocades) with pectolytic and hemicellulolytic activities, Pectinex AFP-L2 (Novo) and Rohapect AP1 with pectolytic main activities, and Rohalase 7069 (Röhm) with cellulolytic activity were used.

Applied enzyme dosages of mash and pomace enzyme preparations were as recommended by the manufacturers for industrial scale. Used enzyme combinations in variants 1−4 were the same as described previously in pilot-scale experiments (*11*). However, due to the high viscosity and water binding, only a 1.5% aqueous suspension of AIS was used in model experiments (yield of AIS was 3%, related to apples). Therefore, also the enzyme dosage was half of that used by Will et al. (*11*).

In detail, the following dosages were used for a 100 mL AIS suspension in mash treatment: 4 μL of Pectinex Smash (variants 1 and 3), 5 μL of Rapidase HP (variant 2), or 5 μL of Rohapect MA plus (variant 4). In pomace treatment, no additional enzyme (variant 1, control), 12.5 μL of Rapidase Pomalique (variant 2), 12.5 μL of Pectinex AFP-L 2 (variant 3), or a combination of 10 μL of Rohapect AP1 and 5 μL of Rohalase 7069 (variant 4) was applied.

Enzymatic Treatments. After the addition of 20 μL of 1 M HCl for adjustment of the pH to ∼3.5, AIS was soaked for 1 h at 20 °C as a 1.5% aqueous suspension (30 mL) and then treated with mash enzymes (dosage diluted in 3 mL of water) for 1 h at 20 °C ("mash treatment") in quadruplicates. The suspension was then immediately centrifuged through a thick cloth (10 min at 4 °C, 1500*g*) to remove the liquid phase. After mash treatment, filtrates (FM) were weighed, adjusted to pH 2.5 with diluted HCl, heated for inactivation of enzymes (10 min at 80 °C), and freeze-dried.

The remaining residues (RM) were either prepared for direct analysis or used for the second enzymatic treatment ("pomace treatment").

For direct analysis, residues were extracted with 25 mL of water during stirring for 5 min, centrifuged, resuspended in 25 mL of EtOH, and heated for 10 min at 75 °C. After removal of EtOH in a Speed-Vac, the residues (RM) were freeze-dried.

Table 1. Composition of the Alcohol-Insoluble Substance from Apples

	amount[a] (%)	*n*
total pectin (galacturonan)	16.30 ± 0.59	$2^b \times 5^c$
EDTA-soluble pectin	10.83 ± 0.31	$2^b \times 3^c$
water-soluble pectin	6.81 ± 0.13	$2^b \times 3^c$
degree of esterification	81.13 ± 0.75	4^c
total dietary fiber	95.54	2^c
soluble dietary fiber	23.19	2^c
insoluble dietary fiber	72.35	2^c
dry matter	96.23 ± 0.11	3^c
ash	0.47 ± 0.01	3^c

[a] Values are mean ± SD. [b] Numbers of repetitions. [c] Numbers of replicates.

For the second enzymatic treatment, residues were resuspended in 30 mL of water and then treated with the cellulase-containing enzyme preparation (dosage diluted in 3 mL of water) for 45, 90, or 120 min at 50 °C and pH 3.5. After centrifugation and inactivation (see above), filtrates (FP) and residues (RP) were obtained after pomace treatment.

Monosaccharide Composition of Polymers in the Filtrates. Freeze-dried filtrates (5 mg) were hydrolyzed with 1 mL of 1 M H_2SO_4 for 5 h at 95 °C under shaking in a thermomixer type 5436 (Eppendorf, Hamburg, Germany). After the hydrolyzed filtrates had cooled to room temperature, ∼190 mg of $Ba(OH)_2 \times H_2O$ was added in solid form for neutralization. Samples were centrifuged to remove insoluble $BaSO_4$ (15 min at 4 °C, 6000*g*). The monosaccharide composition was determined in the supernatant by high-performance anion-exchange chromatography (HPAEC) on a CarboPac PA-100 column (4 × 250 mm) with a corresponding precolumn from Dionex (Idstein, Germany) and a postcolumn addition of 0.5 mL/min of 0.3 M NaOH. The gradient consisted of 0.1 M NaOH, water, and 0.5 M NaOH with a flow rate of 1 mL/min. Monosaccharides were detected by PAD detection using Chromeleon software (Dionex).

Characterization of Molecular Weight Distribution. The molecular weight distribution was determined by gel permeation chromatography (GPC) on a combination of Suprema 100 and Suprema 3000 columns [Polymer Standards Service (PSS), Mainz, Germany] equipped with an HPLC pump 420 from Kontron (Neufahrn, Germany) (flow = 1.0 mL/min; eluent = 0.06 M Na_2HPO_4 and 20% MeOH) and an RI detector from ERC GmbH (Alteglofsheim, Germany) and by using the PSS software Win GPC Scientific V4.02.

Standards with the following elution volumes at peak maximum were used for calibration: glucose (21.3 mL), maltotriose (20.7 mL), and maltopentaose (20.3 mL) (all purchased from Serva) as well as pullulan 5600 (19.5 mL), pullulan 11200 (19.1 mL), pullulan 22000 (18.9 mL), and pullulan 45000 (18.5 mL) (obtained from PSS).

Ten milligrams of freeze-dried filtrate per milliliter was applied to GPC.

Determination of WBC. The WBC was analyzed by using the capillary suction method (*18*). Approximately 10 mg of sample was placed on a glass filter G2 (diameter = 4 cm, pores = 40−90 μm) on a closed chamber filled with water and connected with a graduated capillary (filled with water). The uptake of water by the sample was measured gravimetrically.

RESULTS AND DISCUSSION

Composition of the AIS. From cv. Boskoop apples with skins and cores, a yield of 2.89% AIS was obtained. The composition of the AIS is summarized in Table 1. The galacturonan concentration was ∼17%. Pectin was highly esterified (degree of esterification > 80%). Similar results were described by Voragen et al. (*13*) and Renard et al. (*19*). They found 25 and 27% of galacturonan and degrees of esterification of 75 and 72%, respectively, in their AIS preparations from apples.

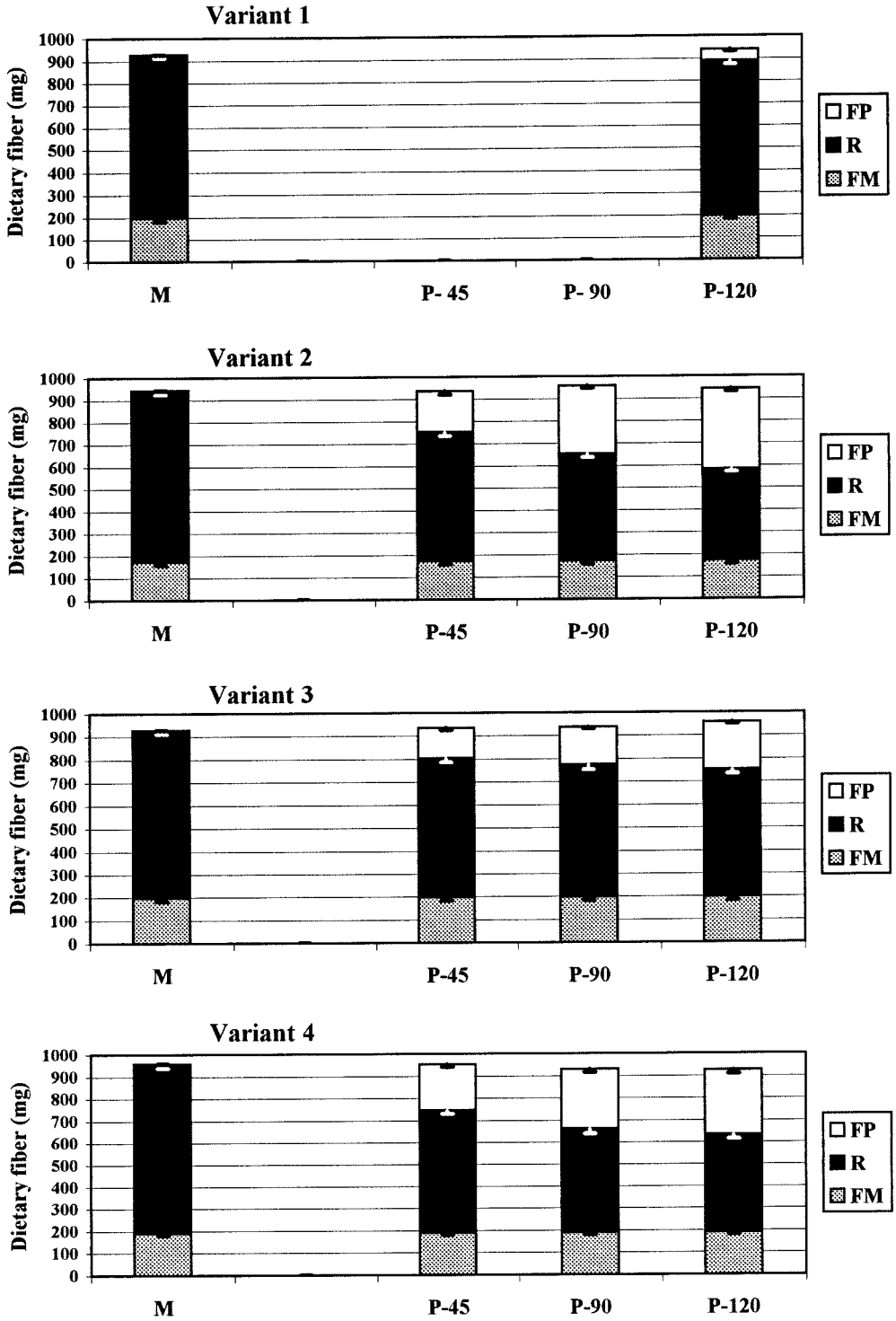

Figure 1. Influence of enzymes on the DF content in filtrates (F) and residues (R) of AIS from apples during mash (M) and pomace (P) treatment (for 45, 90, and 120 min).

The prepared AIS was rich in DF (>95%). More than 70% of the DF was in the insoluble fraction. In an AIS preparation obtained from fresh apples, Renard et al. (*20*) found 63.4% of insoluble and 22.5% of soluble DF. Oligomeric dietary fibers were not detected in our preparation.

DF in Residues and Filtrates. AIS and fractions obtained after enzymatic treatment consisted practically exclusively of DF. The amount of total DF in these fractions was not determined directly, due to the requirement of relatively high sample amounts for the analysis of DF by the AOAC method (*17*). Therefore, the total DF content was indirectly given by the content of dry matter.

Theoretically, 955 mg/g of total DF can be expected as calculated from the composition of AIS (Table 1). As shown in Figure 1, our results are in good correspondence with this theoretical value for both mash and pomace treatments (sum of freeze-dried residues and filtrates). Mash treatment resulted in a DF content between 173 and 198 mg in the filtrates, whereas 730−772 mg of DF was found in the residues.

A further partial degradation of the cell wall occurred during the pomace treatment in variant 1 (control),

Table 2. Content of Galacturonan (GalA) in Filtrates (F) and Residues (R) after Mash (M) and Pomace (P) Treatment of the Alcohol-Insoluble Substance from Apples

	mash treatment[a]			pomace treatment							
	FM[b]	GalA in FM		time	FP[b]	GalA in FP		RP[b]	GalA in RP		total GalA
variant	(mg)	%[c]	mg[c]	(min)	(mg)	%[c]	mg[c]	(mg)	%[c]	mg[c]	(mg)
1	198	40.0 ± 0.7	79.2 ± 1.4	0	0	0	0	730[d]	13.0 ± 0.1	94.9 ± 1.0	174.1
				120	51	57.5 ± 0.6	29.3 ± 0.3	690	9.5 ± 0.2	65.8 ± 1.2	174.3
2	173	26.7 ± 0.5	46.3 ± 0.8	0	0	0	0	772[d]	14.5 ± 0.2	111.9 ± 1.8	158.2
				45	187	48.1 ± 1.1	89.9 ± 2.0	577	4.5 ± 0.1	26.1 ± 0.4	162.3
				90	308	34.9 ± 0.2	107.5 ± 0.5	447	3.4 ± 0.1	15.1 ± 0.5	168.9
				120	364	31.6 ± 0.3	115.2 ± 1.0	408	3.2 ± 0.1	13.9 ± 0.4	175.4
3	198	40.0 ± 0.7	79.2 ± 1.4	0	0	0	0	730[d]	13.0 ± 0.3	94.9 ± 2.3	174.1
				45	134	51.5 ± 0.4	69.0 ± 0.6	603	2.9 ± 0.2	17.6 ± 1.3	165.8
				90	166	42.9 ± 0.3	71.2 ± 0.5	573	3.3 ± 0.3	18.8 ± 1.6	169.2
				120	208	37.4 ± 0.1	77.8 ± 0.3	551	1.7 ± 0.1	9.7 ± 0.4	166.7
4	191	48.1 ± 0.9	91.9 ± 1.7	0	0	0	0	766[d]	9.5 ± 0.2	72.9 ± 1.3	164.8
				45	209	26.6 ± 0.3	55.6 ± 0.6	553	3.7 ± 0.1	20.3 ± 0.8	167.8
				90	271	23.1 ± 0.3	62.5 ± 0.9	468	2.9 ± 0.3	13.7 ± 1.4	168.1
				120	293	22.7 ± 0.1	66.4 ± 0.3	441	2.1 ± 0.1	9.3 ± 0.5	167.6

[a] Mash treatment time = 60 min. [b] mg/g of AIS. [c] Values are mean ± SD (n = 6). [d] After mash treatment.

although no enzymes were added. The amount of DF in the filtrate PF was 51 mg/g of AIS, probably as a result of incomplete removal of mash enzymes during centrifugation. Therefore, the remaining mash enzymes could have been still active under conditions of pomace treatment (2 h at 50 °C). This effect was also observed during apple juice production in technological experiments under the same conditions (*11*). Another point for the appearance of DF in filtrate PF of variant 1 may be the incomplete extraction of soluble material (for instance, pectin) during the single centrifugation after mash treatment.

Pomace treatment (variants 2−4) resulted in an increase of DF in the filtrates PF (Figure 1). The content of DF of the remaining residues decreased with increasing incubation time. The pomace enzyme in variant 2 showed the most intense action on the cell wall material from apples.

The amounts of soluble material found after enzymatic treatment means apple juice of higher yields and quality (*11*). It was reported (*12*) that during pulp enzyming of ground apples lower amounts of total neutral sugars (1.10−1.85 versus 2.80−3.80 g/kg) and uronic acids (1.90−2.70 versus 3.40−3.60 g/kg) were solubilized than during liquefaction. Also, synergistic effects of purified pectolytic and cellulolytic enzymes on the solubilization of saccharides from apple cell wall materials were found by Pilnik (*5*) and Capek et al. (*21*). This clearly shows the efficiency of such enzyme combinations for an effective production of liquid fruit products.

Pectin in Residues and Filtrates. For enzymatic technologies pectin and its partial degradation play a central role in the improvement of yield and quality of apple juice. Therefore, the galacturonan content was determined in fractions obtained after enzymatic mash and pomace treatment. The results are summarized in Table 2. The total galacturonan content was between 158 and 175 mg/g of AIS (theoretical value = 163 mg/g of AIS).

The enzyme formulation in variant 4 was most effective in the release of galacturonan into the filtrate (92 mg/g of AIS) during mash treatment. Residues contained 74−112 mg of galacturonan/g of AIS, indicating that most of the pectin remained in the insoluble fraction of the cell wall after mash treatment.

During pomace treatment, residues obtained after centrifugation were incubated for up to 2 h with pomace enzymes—with the exception of the control experiment in which only water was added (variant 1). Nevertheless, 29 mg of galacturonan per gram of original AIS was found in the filtrate PF of the control. On the other hand, the content of galacturonan decreased in the residue of the control from 95 mg/g of AIS (mash treatment) to 66 mg/g of AIS (pomace treatment) (Table 2).

During the action of pomace enzymes in variants 2−4, the amount of galacturonan increased in filtrates and decreased in residues progressively with the incubation time. In variants 3 and 4, approximately 66 and 78 mg of galacturonan were found in extracts after 2 h, respectively, whereas <10 mg remained in residues as related to 1 g of original AIS. The higher amount of galacturonan in the filtrate FP of variant 2 is due to a lower galacturonan content in the filtrate FM of the same experiment.

Galacturonic acid found in residues after intense enzyme action probably belongs to the "hairy regions" (rhamnogalacturonans) of pectin (*8, 22*).

Changes in Water Binding of Residues. Its ability to bind or hold large amounts of water is an essential functional property of DF. This property plays an important role in the production of functional foods as well as in the gastrointestinal tract. For instance, technological treatment of DF by mechanical or hydrothermal processes may result in an altered water binding (*23−28*). Renard and Thibault (*20*) found WBCs of 14.2 and 7.3 g of H_2O/g of AIS isolated from fresh apples and from depectinated apples pomace, respectively, at pH 3−4 by the capillary suction method.

Besides mechanical methods, enzymatic treatments can also influence water-binding properties of plant cell wall material, through depolymerization of polysaccharides or destruction of naturally grown cellular overstructures. Therefore, we determined the WBC in residues after enzymatic mash and pomace treatment, which served as an appropriate indicator for the intensity of plant tissue destruction.

The WBC of AIS was 14.48 g of H_2O/g. After mash treatment, WBC values were between 10.8 and 12.5 g of H_2O/g of residue RM. In variants 1−4, the following WBC values were found after pomace treatment of 2 h: 9.6 ± 0.4, 7.0 ± 0.3, 7.1 ± 0.1, and 6.9 ± 0.3 g of H_2O/g of residue RP, respectively.

The enzymatic effects on the cell wall material of apples are more prominent if the WBC values are

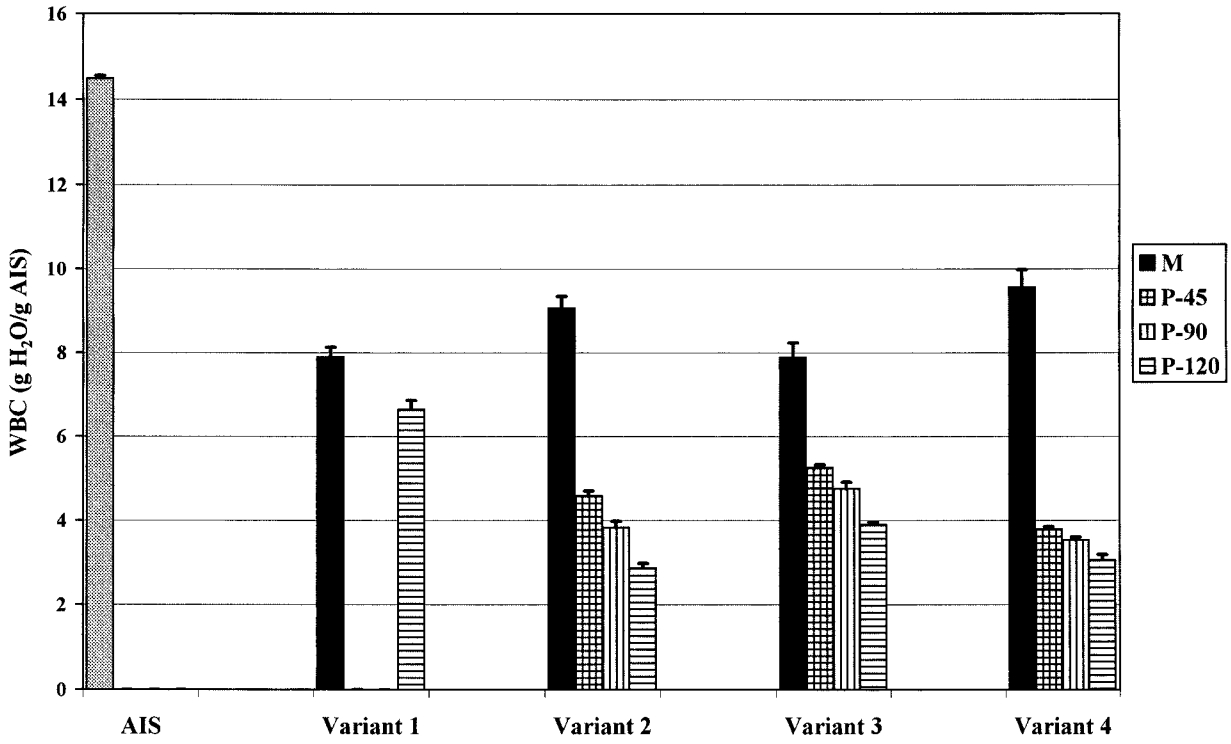

Figure 2. WBC of residues after mash (M) and pomace (P) treatment (for 45, 90, and 120 min) of AIS from apples (values are mean ± SD; $n = 3-5$).

Table 3. Monosaccharide Composition in Hydrolyzates of the Filtrates after Enzymatic Treatment (Variants 1−4) of the Alcohol-Insoluble Substance from Apples[a]

variant	pomace treatment (min)	mg/total filtrate[b]							
		fucose	rhamnose	arabinose	galactose	glucose	xylose	galacturonic acid	total saccharides
1	0	<0.1	4.1	35.0	8.6	8.6	8.0	43.7	108.0
	120	<0.1	1.0	9.8	3.5	4.4	3.9	10.4	33.0
2	0	<0.1	3.2	25.0	6.4	5.1	5.1	50.4	95.2
	45	<0.1	4.3	44.6	14.1	14.8	16.7	41.1	135.6
	90	<0.1	6.7	74.1	24.4	27.4	27.7	108.8	269.1
	120	<0.1	6.9	88.5	28.5	35.3	33.2	122.2	314.6
3	0	<0.1	4.1	35.0	8.6	8.6	8.0	43.7	108.0
	45	<0.1	2.4	29.2	11.3	15.8	15.9	33.0	107.6
	90	<0.1	3.1	28.0	14.6	20.6	19.6	50.7	136.6
	120	<0.1	3.4	49.8	19.2	30.3	26.7	66.0	195.4
4	0	<0.1	3.7	27.5	6.3	2.4	4.4	76.6	120.9
	45	<0.1	2.7	50.1	20.3	36.7	28.8	40.4	179.0
	90	<0.1	3.4	59.1	22.4	43.2	33.4	45.3	206.8
	120	<0.1	5.4	77.6	28.8	66.7	42.9	64.6	286.0

[a] Mash treatment time = 60 min. [b] Related to 1 g of AIS.

related to 1 g of the original AIS (Figure 2). After mash treatment, the WBC was reduced to 7.9−9.6 g of H_2O/g, meaning a decrease of 33.9−45.5%. The strongest effect on the WBC occurred in variants 1 and 3. During pomace treatments, the WBC was progressively reduced by all enzyme preparations with increasing incubation time. The most prominent effects were found in variants 2 and 4, with reductions of WBC of 80.3 ± 0.8 and 78.9 ± 0.9% after 2 h of incubation as compared to AIS, respectively. The smaller effect of Pectinex AFP-L2 is probably due to its lower cellulase activity. In the control experiment, the WBC was reduced to 6.6 ± 0.2 g of H_2O/g.

Monosaccharide Composition of Oligomers and Polymers in Filtrates. After acidic hydrolysis of isolated oligomers and polymers from filtrates, a monosaccharide pattern typically for apple cell wall polysaccharides was obtained. Besides galacturonic acid (GalA), the neutral saccharides arabinose (Ara), glucose (Glc), xylose (Xyl), and galactose (Gal) dominated. Rhamnose (Rha) was also present in all preparations as a typical unit of pectin. Other saccharides such as fucose (Fuc) were found in negligible amounts (Table 3). Both the percentage and the amount of monosaccharide moieties in filtrates are dependent on the spectrum and the activities of the applied enzyme preparations.

A reason for the high release of GalA units into the filtrates during mash treatment is the high pectolytic activity of mash enzymes.

Although no further enzymes were added, 33 mg of saccharides/g of AIS was released into the filtrate of variant 1 after pomace treatment (2 h at 50 °C). Application of pomace enzymes resulted in a strong increase of total saccharides in the filtrates with increasing incubation time. In filtrates of variants 2 and 4, the highest amounts of saccharides were found. The high release of Glc in variant 4 gave a hint of a strong

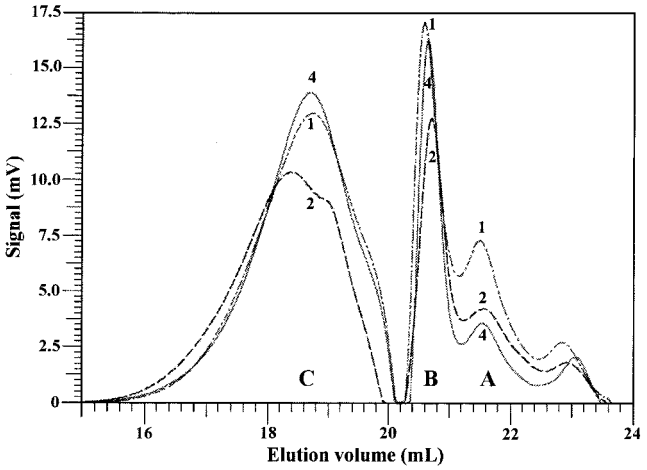

Figure 3. Molecular weight distribution of colloids in filtrates after mash treatment (1, variants 1 and 3; 2, variant 2; 4, variant 4; A, monomer; B, oligomer; C, macromolecular).

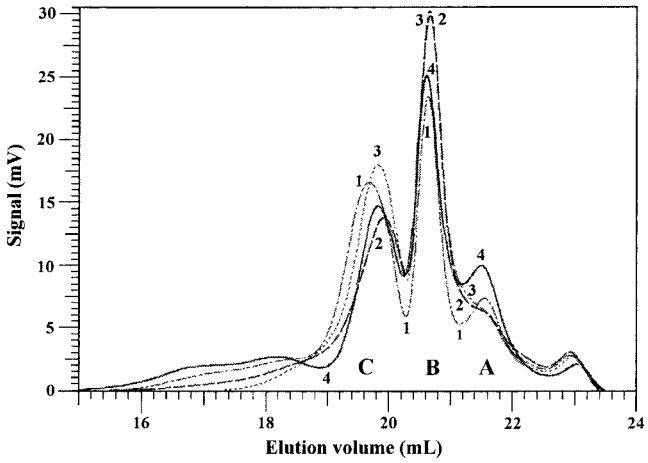

Figure 4. Molecular weight distribution of colloids in filtrates after pomace treatment (1−4, variants 1−4; A, monomer; B, oligomer; C, macromolecular).

cellulose degradation by the cellulolytic enzyme combination (Rohapect AP1/Rohalase 7069).

In detail, higher percentages of GalA (40.5−62.8%) and Rha (3.1−3.8%) were found in filtrates after mash fermentation as compared to pomace treatment (22.6−33.8 and 1.9−2.9%, respectively). On the other hand, in filtrates lower portions of Gal (5.1−10.5%), Glc (2.0−8.0%), and Xyl (4.5−7.4%) were present after mash treatment, whereas 9.8−10.5, 18.5−23.3, and 11.6−15.0%, respectively, were detected in pomace-treated filtrates, except for the filtrate of variant 4 after pomace treatment. GalA was the predominant saccharide unit of oligomers/polymers in all filtrates followed by Ara.

On the other hand, pectic substances isolated from apple juice prepared without enzymatic treatment consisted of >75% of GalA and ∼11% of neutral saccharides (*29*). Voragen et al. (*13*) found that a combination of C_1 cellulase, polygalacturonase, and pectinesterase solubilized 90% of AIS from apples. The main saccharide moieties were Glc, GalA, and Ara.

Molecular Weight Distribution of Polymers in the Filtrates. After mash treatment, three main fractions of different molecular weights were found in the filtrates, consisting of a monomer (A), an oligomer (B), and a macromolecular fraction (C) (Figure 3). The peaks appearing in all chromatograms at an elution volume of 23 mL contained no carbohydrates. Application of enzyme preparation in variant 2 (curve 2) resulted in the lowest amount of oligomers but yielded the highest amount of the high molecular weight fraction (elution volume < 18 mL). A treatment of AIS with Rohapect MA plus (variant 4) released the highest quantity of polysaccharides with a molecular weight of ∼30000 (pullulan standard) in filtrates. The monomeric fraction was maximal when Pectinex Smash (variant 1 and 3) was applied.

Different chromatograms were obtained after pomace treatment of 2 h. The macromolecular fractions were smaller and additionally shifted to lower molecular weights (Figure 4). The elution profile of the variant 1 filtrate was similar to the others. However, the total amount of this filtrate was relatively low (see Figure 1). Highest amounts of monosaccharides appeared in variant 4 (curve 4), whereas oligomers were highest in variants 2 and 3 (curves 2 and 3). In variant 3, the highest amounts of polymers with a molecular weight of <10000 were found, whereas the highest yield of very high molecular weight saccharides was obtained with enzymes of variant 4 (curve 4).

The amounts of oligo- and monosaccharides increased progressively with incubation time during pomace treatment, whereas the polymeric fraction decreased (data not shown).

The breakdown of pectins isolated from apple juices after mash treatment with two commercial pectinases was also determined by Bartolini and Jen (*30*) using high-performance size exclusion chromatography and viscosity measurements. Likewise, Voragen et al. (*13*) found at least three saccharide fractions by GPC including disaccharides and high molecular weight substances released from AIS during the pectolytic and cellulolytic action of different purified enzymes. As in our experiments, the amount and composition of the neutral and acidic saccharides in these fractions depended on the enzymes used or their combinations.

Conclusions. The results of our enzymatic model experiments with an AIS prepared from apples are in good agreement with pilot-scale experiments with apples, in which the same enzyme combinations were applied (*11*). They may lead to a better understanding of processes occurring on the cell wall polysaccharides due to complex enzyme actions during mash and pomace treatments.

Furthermore, it is possible to follow the effects of enzymatic action on the content and composition of DF in liquid fruit and vegetable products, which is of interest for the production of healthy fiber-enriched food products as well as functional food products. For instance, DF fractions isolated after mash and pomace treatment of these juices showed beneficial nutritional effects, for example, a high formation rate of short-chain fatty acids during in vitro fermentation with human fecal flora (*31*).

Alcohol-insoluble substances are a suitable model for the characterization of enzyme actions on fruits and vegetables for the production of juices, macerates, or related products.

ABBREVIATIONS USED

AIS, alcohol-insoluble substance; Ara, arabinose; DF, dietary fiber; F, filtrate; FM, filtrate after mash treatment; FP, filtrate after pomace treatment; Gal, galactose; GalA, galacturonic acid; Glc, glucose; GPC, gel

permeation chromatography; HPAEC, high-performance anion-exchange chromatography; M, mash; P, pomace; PAD, pulsed amperometric detection; R, residue; Rha, rhamnose; RM, residue after mash treatment; RP, residue after pomace treatment; WBC, water-binding capacity; Xyl, xylose.

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