Cell Wall Dissolution during Industrial Processing of Green Beans (*Phaseolus vulgaris* L.)

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Cell wall material was isolated from fresh and industrially processed green beans using two cultivars and two different isolation methods. The chemical composition of the cell wall fractions was compared, and the structure of the water-insoluble polymers was investigated by methylation analysis. This study indicated that the major cell wall polysaccharides of green bean pods were cellulose and pectin, but hemicelluloses were also detected. Major cell wall changes occurred during the sterilization process. Homogalacturonan was partially degraded, and on average 68% of the pectin was solubilized during this process. Sugar analyses indicated that either pectin-associated or hemicellulosic arabinogalactans remained immobilized within the residual cell wall matrix after sterilization. Processing did not affect the overall degree of acetylation. However, the overall degree of methylation decreased after sterilization, most likely predominantly caused by β-eliminative breakdown of highly methylated homogalacturonan regions of the pectin during the heating process.

Keywords: *Cell walls; pectin; processing; Phaseolus vulgaris*

INTRODUCTION

Plant cell walls are the major determinants of textural properties in fruits and vegetables (Ring and Selvendran, 1978, 1981; Selvendran, 1985). Since firmness is an important quality aspect of many economically important plant-based foods, there is a need for a better understanding of the relation between the texture of a plant tissue and chemical properties of the cell walls and middle lamellae.

The physical characteristics of the cell wall and middle lamella primarily depend on the composition and interaction of the constituent polymers. Until now, most research on cell walls has been focused on the composition, structure, and properties of fresh tissues (Seymour et al., 1990; Waldron and Selvendran, 1990; Srisuma et al., 1991; Dawson et al., 1992; Redgwell et al., 1992). In most fruits and vegetables, the cell wall consists typically of 40% pectic polymers, 35% cellulose, 15% hemicellulosic polymers, 5% phenolics, and 5% proteins. In this type of cell wall, these constituent polymers are ordered in three structurally independent but interacting domains (Carpita and Gibeaut, 1993). One domain, the fundamental network of cellulose microfibrils and xyloglucans, is embedded in a second domain of matrix pectic polysaccharides. The third domain consists of structural proteins, such as extensins.

Pectins have been studied frequently because they are thought to be important cell wall components with respect to growth, ripening, and processing of fruits and vegetables. Selvendran supposed that pectins of the middle lamella are highly methyl esterified and slightly branched (Selvendran, 1991). Cell wall pectin, however, consists of two distinguishable regions, a linear homogalacturonan and a branched rhamnogalacturonan (De Vries et al., 1982). There is good evidence for covalent cross-linking of pectins via ester bonds, connected either to other pectic molecules, hemicellulose, cellulose, or protein. Fry (1986) suggested that at least some of these are diferulate bridges between adjacent pectin molecules. The additional pectin is connected to the wall only by its ability to form calcium intermediated noncovalent gels (Jarvis, 1984). For legumes, the overall monosaccharide composition of cell walls of *Phaseolus vulgaris* (green beans) was shown to be similar to that of *Phaseolus coccineus* (runner beans) (O'Neill, 1980; Ryden and Selvendran, 1990a; O'Neill and Selvendran, 1983, 1985; Selvendran and King, 1989). In *P. coccineus* two types of xyloglucan, with different degrees of branching, can be discriminated. In addition, complexes of xylans with pectic material have been extracted (Ryden and Selvendran, 1990a). Parenchymatous cell walls of *P. coccineus* typically contain a high level of hydroxyproline-rich glycoproteins.

Until now, research on the effect of processing on the chemical structure of cell walls has been limited to determinations of total pectin and neutral sugar content (Sistrunk and Cain, 1960; Sistrunk et al., 1989). Several studies revealed that the major cleavage reaction leading to vegetable softening was a β-eliminative depolymerization of intercellular pectin. Increased methyl ester content resulted in a higher rate of pectin degradation (Sajjaanantakul et al., 1989). Some cations and anions were also shown to enhance β-elimination (Keybets and Pilnik, 1974; Van Buren and Peck, 1981; Van Buren, 1986; Van Buren et al., 1990; Sajjaanantakul et al., 1993). However, possible modifications of minor but important cell wall constituents remain largely unknown. This study focused on chemical cell wall changes occurring during processing of green beans. We report the sugar composition, acetyl groups, methyl esters, and sugar linkages of cell wall polymers isolated from fresh, blanched, and sterilized green beans.

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MATERIALS AND METHODS

Plant Material and Processing Conditions. Green beans (*P. vulgaris* L.) cv. Montano (fresh market bean) and cv. Masai (industrial market bean) were grown at the experimental research station PAGV (Lelystad, The Netherlands) and harvested at edible maturity at 68 and 77 days after sowing, respectively. The pods were cut into parts of 3–4 cm in length and blanched at 90 °C for 4 min. For the canning process, portions of 410 g were packed into cans (720 mL) and a 0.25 M NaCl solution (brine) was added. Closed cans were sterilized at 118 °C for 30 min, cooled, and stored at 15 °C for 2 weeks. The pH of the brine after sterilization was 5.5. Quadruplicate samples were taken from fresh, blanched, and sterilized beans. Each sample was cryomilled in liquid nitrogen by using a Moulinex food processor. All samples were stored at −20 °C until further analysis.

Firmness Measurements. The firmness of the sterilized bean pods was measured in triplicate using an Instron Universal Testing Machine equipped with a Kramer shear cell. Forty grams of material was placed in the cell with the length axis of the pods perpendicular to the openings of the shear cell. The maximal force (top value) applied with the shear press to break through the beans was used to quantify the firmness of the beans.

Isolation of Cell Wall Material. *(a) Alcohol-Insoluble Residue (AIR).* Fifty grams of frozen material was immersed in 100 mL of cold (−30 °C) ethanol (96% v/v), homogenized with an ultraturrax by four bursts of 1 min, and collected on a Whatman GF/C filter. The material was suspended in 50 mL of ice-cold aqueous ethanol (70% v/v) and stirred for 1 h at 2 °C. The material was filtered again, washed twice with 50 mL of 100% acetone until the filtrate was colorless, and dried overnight to yield the AIR.

(b) Water-insoluble residue (WIR) was isolated according to a slightly modified method of Selvendran et al. (1985). Fifty grams of frozen material was homogenized with an ultraturrax by four bursts of 1 min in 100 mL of SDS (1.5% w/v), 5 mM $Na_2S_2O_5$, and 20 mM HEPES (pH 7.5). This slurry was centrifuged at 10 °C for 15 min at 2500 g. The pellet was suspended in 75 mL of SDS (0.5% w/v), 3 mM $Na_2S_2O_5$, and 20 mM HEPES (pH 7.5) and stirred overnight at 4 °C. Next, this slurry was centrifuged using the conditions as mentioned above. The pellet of this centrifuge step was washed three times with distilled water. After exhaustive dialysis, the residue was lyophilized and yielded the water-insoluble residue. The supernatants of the two consecutive centrifugation steps were combined and concentrated by ultrafiltration using an Amicon cell equipped with a PM 10 membrane. Four volumes of ethanol was added to the concentrates to precipitate the polymers overnight at −20 °C. Precipitates were recovered by centrifugation as mentioned above and lyophilized to yield the water-soluble polymer (WSP) fraction. All cell wall fractions were milled in a Retsch ball mill prior to further analysis.

Monosaccharide Composition. Each sample was analyzed in duplicate. Sugars were released from the samples by dispersing in cold 11.5 M H_2SO_4 for 2 h at 20 °C, followed by hydrolysis in 1 M H_2SO_4 for 2 h at 100 °C (Seaman hydrolysis). The hydrolysates were filtered through a Whatman GF/C glass fiber filter and neutralized with $BaCO_3$. Samples (10 mL) of the neutralized hydrolysates were analyzed for neutral sugars by using a HPLC system (Pharmacia LKB low-pressure mixer, HPLC pump 2248, and autosampler 2157) equipped with a Carbopack PA1 column (250 × 4 mm, Dionex). The eluents, consisting of Milli Q water and 150 mM NaOH, were sparged and pressurized with helium. Prior to injection, the system was equilibrated with 30 mM NaOH for 8 min at a flow rate of 1.0 mL/min at ambient temperature. At 0.1 min after injection, the eluent was shifted from 30 mM NaOH to Milli Q water. After each run, the column was regenerated with 150 mM NaOH for 15 min. Compounds were detected with a Dionex pulsed amperometric detector fitted with a gold working electrode. The applied potentials were set at $E_1 = 0.1$ V, $E_2 = 0.6$ V, and $E_3 = -0.6$ V against a Ag/AgCl reference electrode. Pulse durations for the applied potentials were 500, 100, and 50 ms, respectively. Anhydrouronic acids were determined as described by Ahmed and Labavitch (1977).

Table 1. Firmness[a] (Newtons) of Green Bean Cultivars Montano and Masai

treatment	cultivar	
	Montano	Masai
fresh	3851 ± 250[a]	3712 ± 271[a]
blanched	3132 ± 40[b]	3079 ± 60[b]
sterilized	158 ± 4[c]	155 ± 3[c]

[a] The mean values are significantly ($P < 0.05$) different for treatment and/or cultivar if followed by a different letter ($n = 4$, ± SD).

Starch Content. To solubilize starch, 5 mL of HCl (8 M) and 20 mL of dimethyl sulfoxide were added to 250 mg of sample. After an incubation period of 60 min in a water bath, which contents were shaken, 5 mL of NaOH (8 M) and citrate buffer (Titrisol/pH 4, Merck 9884) were added to a final volume of 100 mL. After filtration, 0.1 mL of sample was used to quantify the starch content using test combination catalog no. 207748 from Boehringer Mannheim.

Protein Content. The nitrogen content of the CWM fractions was measured using a Carlo Erba CHNS-OEA 1108 elemental analyzer. The protein content was estimated by multiplying the nitrogen value by 6.25.

Methyl and Acetyl Substituents. The amount of methyl and acetyl groups was determined by using a HPLC system under the conditions described previously by Voragen et al. (1986).

Methylation Analysis. WIR samples were methylated using a modified method of Ciucanu and Kerek (1984). WIR (5 mg) was cryomilled with a SPEX freezer mill and dispersed in anhydrous dimethyl sulfoxide (1 mL) by occasional sonification for up to 72 h prior to the methylation procedure. After that addition of 20 mg of powdered NaOH and 0.5 mL of CH_3I, the solutions were stirred overnight. The excess of CH_3I was evaporated in a stream of air. The methylated samples were subsequently dialyzed using running tap water (16 h), followed by distilled water (6 h). Next, the samples were lyophilized. The lyophilized powder was methylated again starting with dispersion in anhydrous dimethyl sulfoxide. Hydrolysis was performed at 121 °C in TFA (2 M). Partially methylated sugars were reduced with $NaBD_4$ and acetylated according to the method of Englyst and Cummings (1984). The partially methylated glycitol acetates were separated and quantified using a fused silica capillary column (30 m × 0.255 mm, wall coated with DB-1701, 1.0 mm) in a GC system (Carlo Erba HRGC 5300 Mega series) equipped with a FID. The sample composition was calculated using effective carbon response (ECR) factors (Sweet et al., 1975). Identification was confirmed by GC–MS (Carlo Erba Mega series coupled to a Carlo Erba QMD 1000 MS equipped with a fused silica column coated with CPSIL 5 CB, 25 m × 0.25 mm, 0.12 mm). The 3,4- and 2,3-*O*-methylated and, separately, the 2- and 4-*O*-methylated xylitol acetates coeluted and their relative amounts were calculated from the relative abundance of the ions at m/z 117 and 118, respectively.

Lignin Content. The presence of lignin was studied by staining transverse sections with phloroglucinol/HCl and subsequent examination by light microscopy (Waldron and Selvendran, 1990).

Statistical Analysis. Data sets were subjected to analysis of variance (ANOVA, Genstat 5) to determine least significant differences (lsd) among cultivars and processing treatments. All analyses were carried out in duplicate, and the entire experiment was repeated four times.

RESULTS

Firmness of Beans. The firmness values, as measured with an Instron, of both green bean cultivars were comparable and decreased during processing (Table 1). The strongest decrease occurred during the sterilization treatment.

Table 2. Amounts[a] of Dry Weight (DW), Alcohol-Insoluble Residue (AIR), Water-Insoluble Residue (WIR), and Soluble Material (WSP) of Green Bean Cultivars Montano and Masai

treatment	DW, mg/g of fresh	AIR, mg/g of DW	WIR, mg/g of DW	WSP, mg/g of DW
		Cv. Montano		
fresh	100 ± 1[a]	582 ± 3[a] (273)	389 ± 82[a,b] (242)	125 ± 13[a] (13)
blanched	105 ± 4[a]	637 ± 37[a,b] (314)	407 ± 80[a,b] (265)	191 ± 23[b,c] (15)
sterilized	87 ± 3[b]	691 ± 53[b] (319)	490 ± 45[b] (239)	214 ± 49[c] (61)
		Cv. Masai		
fresh	85 ± 1[b]	584 ± 6[a] (310)	390 ± 58[a,b] (252)	164 ± 13[a,b] (10)
blanched	85 ± 2[b]	595 ± 49[a] (309)	362 ± 4[a] (245)	185 ± 10[b,c] (22)
sterilized	71 ± 4[c]	671 ± 39[b] (351)	448 ± 60[a,b] (247)	231 ± 30[c] (81)

[a] Numbers in parentheses represent the amount of cell wall sugars. The mean values in a column are significantly ($P < 0.05$) different for treatment and/or cultivar if followed by a different letter ($n = 4$, ± SD).

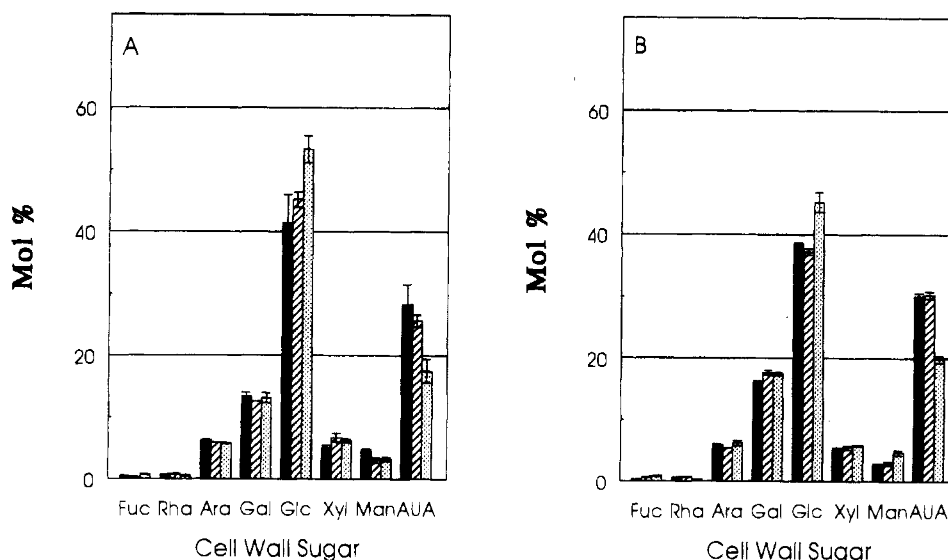

Figure 1. Neutral sugar composition and uronic acid content of AIR from green bean cultivars (A) Montano and (B) Masai. (Solid bars) fresh; (striped bars) blanched; (dotted bars) sterilized.

Isolation of Cell Wall Material. Cell wall material (CWM) was isolated from two green bean cultivars using two different methods. First, AIR was isolated, which contained all CWM in addition to proteins, starch, and other high molecular weight components. In addition, the plant material was extracted with SDS buffers, which resulted in an immediate separation of water-soluble (WSP) and water-insoluble cell wall polymers (WIR). The amounts of cell wall materials and dry weights of the different fractions are summarized in Table 2. During sterilization, the intercellular spaces of the bean pods become filled with brine solution, which resulted in a lower dry weight as compared with the fresh samples. The relative proportion of CWM was higher in the sterilized samples as compared with fresh samples since small molecules and salts leach out into the brine. Additionally, heat treatment denatures proteins, thereby affecting the overall composition of the WIR. Almost all protein could be extracted from fresh and blanched samples with the SDS buffer, while, after sterilization, most proteins were unextractable and were recovered in the WIR.

Protein accounted for 19–24% of the weight of all AIR and WIR samples of sterilized material. WIR of fresh and blanched samples contained approximately 5–6% protein. In contrast, the WSP fractions contained approximately 70% protein. The AIR and WIR of cv. Montano beans contained 23–30% starch, whereas the AIR and WIR of cv. Masai beans contained 13–15% starch.

CWM accounted for 46–53% of the AIR from both cultivars. Processing resulted in an increased solubilization of CWM from both cultivars, reflected by an increase in contents of the WSP fractions. However, these polymers did not leach out into the brine but remained entangled inside the beans. The amount of polymers in both the blanching water and the brine appeared to be negligible. For this reason no attempts were made to analyze the blanching water and brine in detail.

Analysis of Carbohydrate Composition. A comparison of the cell wall fractions during processing revealed that sterilization drastically altered the overall sugar composition of the cell walls of both cultivars (Figures 1 and 2). After this treatment, the total uronic acid content of the AIR and WIR + WSP was reduced to 34–38%, as can be calculated from the molar sugar composition and the yields of the different fractions (Table 2; Figures 1 and 2). This reduction was not accompanied by a loss of other pectic sugars such as arabinose and galactose. In the WIR there was an additional decrease in pectic sugars, mostly uronic acid, but substantial amounts of galactose and arabinose were also lost. Since the latter pectic sugars were recovered in the WSP fraction, sterilization apparently solubilized these compounds. The overall cell wall compositions of both cultivars were identical, but galactose seemed to be more abundant in the CWM of cv. Masai beans.

Degree of Methylation and Acetyl Substituents. Similarly to the carbohydrate composition, sterilization affected the degree of methylation (DM) of the cell wall fractions. The DM of the AIR of sterilized beans was on average 14% lower as compared with the same

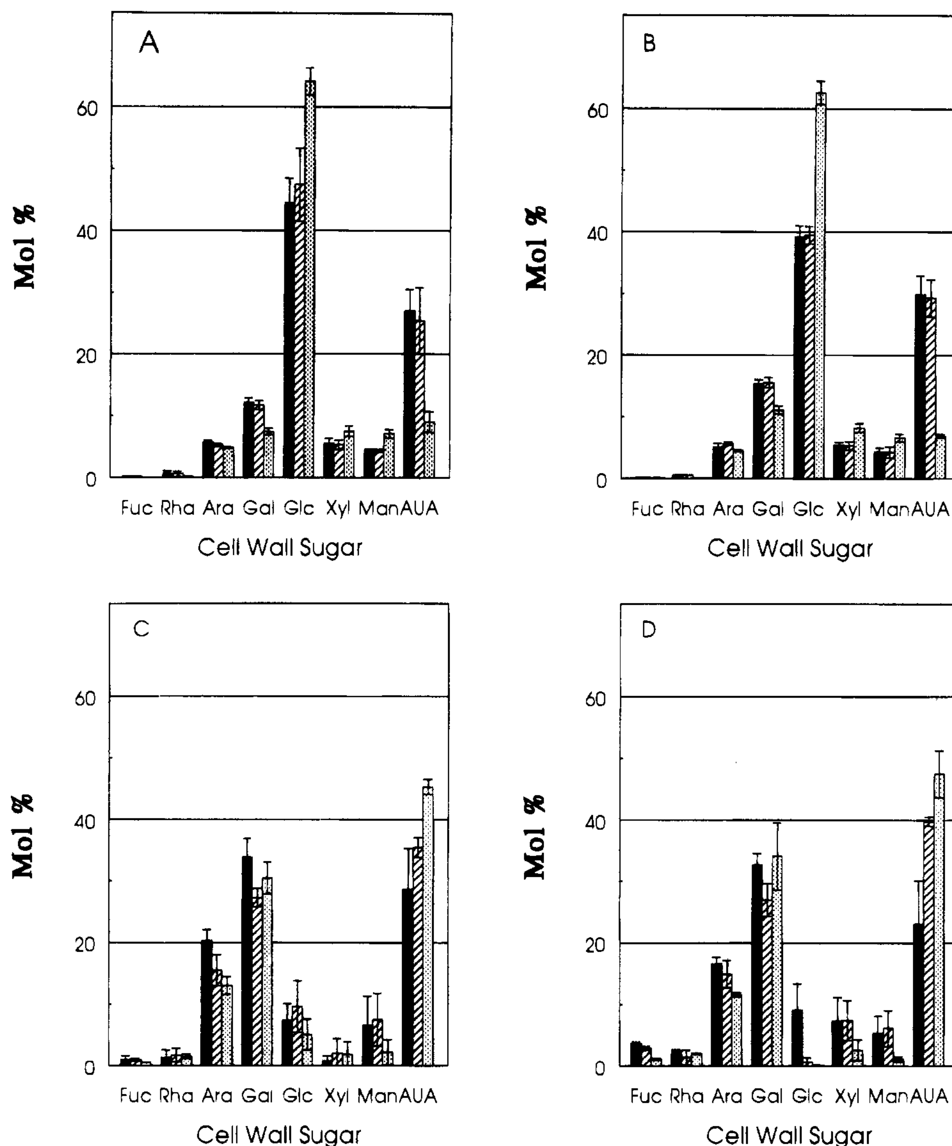

Figure 2. Neutral sugar composition and uronic acid content of WIR and WSP from green bean cultivars Montano and Masai: (A) Montano WIR; (B) Masai WIR; (C) Montano WSP; (D) Masai WSP. (Solid bars) fresh; (striped bars) blanched; (dotted bars) sterilized.

Table 3. Degree of Methylation[a] (Mole Percent of Uronic Acid Content) of Cell Wall Fractions from Green Bean Cultivars Montano and Masai during Processing

treatment	AIR	WIR	WSP
	Cv. Montano		
fresh	50.6 ± 1.0[a]	34.0 ± 0.9[a]	55.8 ± 12.8[a,b]
blanched	49.9 ± 1.0[a]	45.6 ± 3.5[b]	69.5 ± 9.9[a,b]
sterilized	36.8 ± 2.0[b]	31.1 ± 7.6[a]	46.1 ± 6.7[a]
	Cv. Masai		
fresh	51.7 ± 3.8[a]	33.4 ± 1.5[a]	57.9 ± 13.4[a,b]
blanched	49.1 ± 4.0[a]	44.4 ± 4.7[b]	49.1 ± 3.9[a]
sterilized	37.7 ± 0.7[b]	27.3 ± 0.5[a]	40.9 ± 3.8[a]

[a] The mean values in a column are significantly ($P < 0.05$) different for treatment and/or cultivar if followed by a different letter ($n = 4$, ± SD).

Table 4. Amount of Acetyl Groups[a] (Micromoles per Gram of DM) in Cell Wall Fractions from Green Bean Cultivars Montano and Masai during Processing

treatment	AIR	WIR	WSP
	Cv. Montano		
fresh	124 ± 5[a]	105 ± 9[a,b]	6 ± 3[a]
blanched	125 ± 1[a]	108 ± 5[a,b,d]	9 ± 3[a]
sterilized	121 ± 3[a]	83 ± 1[c]	33 ± 6[b]
	Cv. Masai		
fresh	139 ± 1[b]	125 ± 12[d]	9 ± 2[a]
blanched	144 ± 7[b]	119 ± 10[b,d]	10 ± 1[a]
sterilized	145 ± 5[b]	91 ± 7[a,c]	50 ± 12[c]

[a] The mean values in a column are significantly ($P < 0.05$) different for treatment and/or cultivar if followed by a different letter ($n = 4$, ± SD).

samples of blanched and fresh beans (Table 3). In contrast, the DM of WIR of fresh beans was almost as low as measured for WIR of sterilized samples. The DM of the WSP fractions was higher as compared with the WIR samples. The total amount of acetyl substituents remained constant; 30% of the acetyl groups was located in the WSP fractions after processing (Table 4).

Methylation Analysis. The method of Ciucanu and Kerek (1984) for methylation of mono- and disaccharides has been widely adopted to analyze complex carbohydrates. Needs and Selvendran (1993) recently concluded that the undesirable oxidative degradation as noted by York et al. (1990) could easily be overcome by addition of sodium hydroxide prior to the addition of methyl iodide. The results obtained by these authors indicated almost complete methylation.

The methylation analyses performed in this study were not quantitative (Table 5). This is based on the

Table 5. Abundance of Sugar Linkages in Mole Percent in WIR Samples from Fresh, Blanched, and Sterilized Green Bean Cultivars Montano and Masai

	cv. Montano			cv. Masai		
linkage site	fresh	blanched	sterilized	fresh	blanched	sterilized
fucose						
terminal	0.4	0.4	0.3	0.5	0.6	0.4
rhamnose						
1,2	0.1	0.1	0.1	0.1	0.4	0.1
1,2,4	0.2	0.3	0.1	0.3	0.3	0.2
arabilnose						
terminal	1.4	1.3	1.0	1.4	1.7	1.2
1,5	4.7	4.5	2.7	2.8	3.2	3.5
1,3,5	0.8	0.8	1.0	0.9	1.0	1.5
arabinitol	0.9	0.6	0.4	1.4	1.8	2.2
galactose						
terminal	1.2	1.1	0.6	1.5	2.0	1.5
1,2	0.1	0.1	0.1	0.3	0.2	0.3
1,4	3.3	3.3	2.8	4.1	3.9	3.6
1,3,6	0.1		0.1	0.2	0.2	0.1
1,4,6	0.3	0.3	0.3	0.5	0.8	0.8
1,3,4	0.8	0.7	0.7	0.7	0.7	1.9
1,2,4	0.6	0.6	0.7	0.7	0.5	0.8
galactitol	0.1	0.1	0.1	0.3	0.3	0.7
glucose						
terminal	1.7	0.5	1.6	1.7	1.9	2.1
1,4	67.2	69.4	76.9	63.0	55.8	52.1
1,4,6	3.6	3.9	4.1	4.7	5.3	5.7
1,3,6	0.1	0.1			0.5	0.2
glucitol	4.1	1.6	1.0	1.6	2.7	4.3
xylose						
terminal	1.1	1.0	0.9	1.6	1.9	1.7
1,2	0.6	0.9	0.7	1.0	1.1	1.2
1,4	1.6	2.3	1.5	2.6	3.0	3.3
1,2,4	0.8	0.6	0.5	1.3	1.5	1.4
1,2,3	*a*	*a*	*a*	*a*	*a*	*a*
1,3,4	*a*	*a*	*a*	*a*	*a*	*a*
xylitol	0.8	0.9	0.2	1.4	1.7	2.8
mannose						
terminal	0.6	1.1	0.6	0.0	0.1	0.1
1,4	2.3	2.1	1.1	3.4	3.8	3.8
mannitol	0.5	0.4	0.2	0.4	0.6	1.2

[a] Not quantified, only detected on GC−MS.

low recoveries observed for galactose in comparison with the Saeman hydrolysis without methylation. The bulk of the glucosyl residues were (1−4)-linked, with significant amounts of (1−4,6)-linked and terminally linked residues. Minor amounts of (1−3,4)- and (1−2,4)-linked residues were also detected. Galactosyl residues were mainly (1−4)-linked, although terminally linked and (1−2)-linked residues were also detected. Xylosyl residues were mostly (1−4)-linked, but minor amounts of (1−2)-, (1−2,4)-, and (1−3,4)-linked and terminally linked xylosyl residues were also present. Arabinosyl residues were mainly (1−5)- and (1−3,5)-linked and terminally linked. Rhamnosyl residues were present in small amounts and appeared to be (1−2)- and (1−2,4)-linked. The detected fucosyl residues were only terminally linked.

DISCUSSION

Cell Wall Constituents. Analysis of the AIR and WIR showed that the cell walls of fresh green beans contain cellulose and pectic polysaccharides. Hemicellulosic polymers are also present, however, to a lesser extent as can be concluded from the xylose and mannose content (Figures 1 and 2). This type of cell wall composition is characteristic for parenchyma, which is the major tissue type in pods of green beans (Reeve and Brown, 1968).

When the cell wall compositions of both cultivars were compared, it was observed that cell walls from cv. Montano beans contained less galactose than cell walls from cv. Masai beans. Previously, it has been shown for kiwi and nectarines that levels of galactose decreased during fruit ripening (Dawson et al., 1992; Redgwell et al., 1992). The discrepancy between the two cultivars might therefore be due to harvesting at different developmental stages. On the other hand, it cannot be excluded that the galactose levels are cultivar specific and play a role in determining textural firmness after processing.

For the WIR of fresh samples, it was observed that the degree of methylation was very low in comparison to the AIR of fresh samples (Table 3). This low value might be explained by pectin methylesterase (PE) activity during the first homogenization step. At low temperatures PE might still be active as was previously shown by isolation procedures of tomato fruit cell walls (Koch and Nevins, 1989). However, for the WIR of blanched samples, the DM was not significantly different as compared with the AIR of blanched samples (Table 3). This latter result indicates that no residual enzyme activity was present after blanching. The soluble fraction (WSP), obtained during WIR preparation, contained mostly pectin, with a relatively high degree of methylation as compared with the WIR of the same samples. It is not possible to calculate a degree of acetylation for the AIR and WIR samples, because acetyl groups can be linked to pectin as well as to certain hemicelluloses. The WSP fractions, however, consist mainly of pectin (Figure 2), and the degree of acetylation of these soluble pectins can be calculated to be approximately 20−30%.

To obtain insight into the types of polymers contributing to the textural aspects of the tissue, a methylation analysis is performed. The bulk of the (1−4)-linked glucose residues arise from cellulose and starch. Also, the bulk of the (1,4,6)-linked glucose residues are likely to be derived from starch. Removal of starch with 90% DMSO was omitted since preliminary experiments had indicated that appreciable amounts of pectins were coextracted with 90% DMSO after sterilization. The bulk of the terminally linked and (1−5)-linked arabinitol in all fractions most probably originated from side chains of rhamnogalacturonan. The formation of 2,3,5-tri-*O*-methylarabinose derivatives shows that arabinose is mainly present in the furanoid form. All other cell wall sugars are assumed to be present in the pyranoid form. Most xylose residues are (1,4)-linked. Xylans are the traditional well-known origin of (1−4)-linked xylosyl residues and are the main hemicelluloses found in secondary walls of dicotyledonous plants (Brett and Waldron, 1990). Secondary walls, however, are only abundant in the hypodermal fiber layer of the bean pod (Reeve and Brown, 1968); consequently, xylans most likely are elements of the primary walls of *P. vulgaris*. Ryden and Selvendran (1990), however, proposed that, in addition to the common (1,2)-linked, some (1,4)-linked xylitol residues are an integral part of the xyloglucan of *P. coccineus*, which resembled the overall cell wall composition of *P. vulgaris* very well (O'Neill and Selvendran, 1980). Evidence for the occurrence of (1−4)-linked xylosyl residues in isolated xyloglucan was also obtained by Karacsonyi and Kovacik (1989) in cell walls from suspension-cultured popular cells. In general, methylation of polysaccharides containing high levels of uronic acids is not efficient and may be complicated due to degradation. A low recovery of galactose especially was observed when the results of the methylation analysis were compared with the results of Saeman

hydrolysis. This low recovery of galactose might be explained by β-eliminative breakdown of pectins carrying short side chains of neutral sugars. These segments might be lost during dialysis of the methylated samples (Ryden and Selvendran, 1990b; Waldron and Selvendran, 1990). However, the analysis rendered sufficient data to outline the general features of the neutral cell wall polymers.

Cell Wall Changes during Processing. In general, cell wall changes occurring during processing can be a result of enzymatic and chemical reactions. In this study, only a high blanching temperature, causing rapid enzyme inactivation, was applied and cell wall changes were expected to be of chemical nature only. Our analyses showed that major alterations occurred during the sterilization of green beans (Figures 1 and 2). It was observed that during this treatment approximately 20% of galacturonite fragments were degraded into small fragments, which were not retrieved in our cell wall isolations. Since also the overall degree of methylation was lower after sterilization, this fraction was most likely highly methylated, poorly branched, and acetylated, suggesting that this pectin originated from the middle lamellae (Sweet et al., 1975; Ryden and Selvendran, 1990b). Sterilization presumably results in degradation of the middle lamellar pectin, thereby reducing the interaction between cells. Recently, a similar observation was made using cryo-SEM of potato cells during steam cooking (Marle et al., 1992).

Analysis of the WIR and soluble fractions revealed that pectins, as indicated by uronic acid content, were solubilized in the cell wall during the sterilization process. These pectins did not leach out into the brine but remained entangled in the cell walls inside the bean pods and were therefore recovered in the WSP fraction. However, 21–28% of the uronic acids and 70–80% of the galactose and arabinose residues remained insoluble. Arabinose and galactose are characteristic for branched rhamnogalacturonans but also occur in association with xyloglucan (O'Neill and Selvendran, 1983, 1985; Carpita and Gibeaut, 1993). Since the methylation analysis (Table 5) showed that the arabinosyl and galactosyl residues were typically (1,5)- and (1,4)-linked, respectively, these sugars were most likely derived from highly branched pectins with type I arabinogalactan side chains. It can, however, not be excluded that, after sterilization, separate arabinan, galactan, or arabinogalactan polymers remain embedded within the cell wall matrix. Separate arabinogalactans were isolated from pea cell walls and were thought to constitute a second matrix layer around the cellulose microfibrils (Talbott and Ray, 1992).

In conclusion, two major effects of sterilization on the pectin of cell wall and middle lamella can be discriminated. First, linear homogalacturonan, presumably originating from the middle lamella, is degraded. Second, rhamnogalacturonan is partially solubilized, probably by breakdown of some covalent linkages. These solubilized polymers, however, remained entangled within the cell wall matrix, probably due to the branched characteristics. It can be envisaged that due to the breakdown as well as the solubilization of the pectins within the cell wall the contribution of these polymers to the firmness of the cell wall is reduced. This results in a less rigid cell wall.

To investigate the interaction of pectins with other cell wall components in more detail, different pectin and hemicellulose fractions are currently being characterized. These studies will also indicate whether alterations of other cell wall compounds during processing are of importance in determining the textural firmness of processed green beans.

ABBREVIATIONS USED

AIR, alcohol-insoluble residue; WIR, water-insoluble residue; WSP, water-soluble polymers; CWM, cell wall material; DW, dry weight; TFA, trifluoroacetic acid; SDS, sodium dodecyl sulfate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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