# Changes in Pectic and Hemicellulosic Polymers of Green Beans (*Phaseolus vulgaris* L.) during Industrial Processing

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Pectic and hemicellulosic material was extracted from the alcohol-insoluble residues of two fresh and industrially processed green bean varieties. The carbohydrate compositions and relative molecular masses of these fractions were determined. Fresh beans of cv. Odessa contained more pectin and hemicellulose but less cellulose than cv. Masai. The major changes occurred during sterilization and concerned the pectic polymers. In fresh beans the major part of the pectins was most likely covalently linked to other cell wall polymers since they were extractable only after mild saponification of the cell wall. Sterilization resulted in degradation and solubilization of pectic polymers from the cell wall and middle lamella. The galacturonic acid backbone was partially degraded. Side chains, however, were not removed from the pectic polymers, resulting in an accumulation of branched pectins of low molecular mass in the buffer-soluble fraction. The amount of cyclohexane-*trans*-12,-diaminetetraacetate-soluble material showed a small increase after blanching, but the amount after sterilization was the same as compared with the fresh material. These results confirm the hypothesis that pectins are solubilized by degradation of the galacturonic acid backbone during industrial processing. The changes in pectic polymers during processing are summarized in a scheme. No significant changes were observed in the hemicellulose and cellulose fractions.

**Keywords:** *Cell walls; pectin; hemicellulose; processing; Phaseolus vulgaris; texture; dietary fiber*

## INTRODUCTION

Texture is a major quality attribute that contributes to the consumer acceptance of fruits and vegetables. Processing of fruits and vegetables is generally accompanied by softening of the plant tissue. Blanching and sterilization affect plant tissues by destroying turgor, swelling the cell walls, and macerating the tissue (Van Marle et al., 1992). As a result, the texture of processed vegetables can be attributed mainly to the structural integrity of the cell wall and middle lamella (Jackman and Stanley, 1995). Recent cell wall models envision a cellulose−hemicellulose structural domain embedded in a second domain consisting of pectic substances, while a third domain contains covalently cross-linked proteins (Carpita and Gibeaut, 1993). Pectic polymers are key substances determining the mechanical strength of the primary cell wall and adhesion between cells. Therefore, pectins have been the main subject of studies aimed at elucidating the cell wall changes occurring during processing. Unfortunately, most of this work has been limited to quantitative determinations of overall degraded pectins, without more qualitative studies concerning the origin and type of pectins being released (Sistrunk and Cain, 1960; Sistrunk et al., 1989). Several studies revealed that the major cleavage reaction leading to vegetable softening was a $\beta$-eliminative depolymerization of methylesterified pectin (Sajjaanantakul et al., 1989). Some cations and anions were shown to enhance $\beta$-elimination (Keijbets and Pilnik, 1974; Van Buren and Peck, 1981; Van Buren, 1986; Van Buren et al., 1990; Sajjaanantakul et al., 1993).

In a previous study we reported on the overall cell wall changes occurring during processing of green beans (Stolle-Smits et al., 1995). Two major effects of sterilization on the pectin of cell wall and middle lamella were discriminated. First, linear homogalacturonan, presumably originating from the middle lamella, was degraded. Second, branched rhamnogalacturonan was partially solubilized. These solubilized polymers, however, remained entangled within the cell wall matrix, probably due to their branched characteristics. We now report in more detail on the changes observed in pectic and hemicellulosic polymers extracted from cell walls during different stages of industrial processing.

## MATERIALS AND METHODS

**Plant Material and Processing Conditions.** Green bean (*Phaseolus vulgaris* L.) cvs. Masai and Odessa were grown in a greenhouse and harvested at edible maturity (i.e. approximately 20 days after flowering). 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 glass jars (720 mL) and a 0.25 M NaCl solution (brine) was added. Closed glass jars were sterilized for 30 min at 118 °C, cooled, and stored for 2 weeks at 15 °C. The pH of the brine after sterilization was 5.5. Triplicate samples were taken from fresh, blanched, and sterilized beans. The green beans were split lengthwise, and the seeds were removed because they were very different in composition and did not contribute to the texture of these green beans. The pods were cryomilled in liquid nitrogen by using a food processor (Moulinex Masterchef 20). All samples were stored at −20 °C until further analysis.

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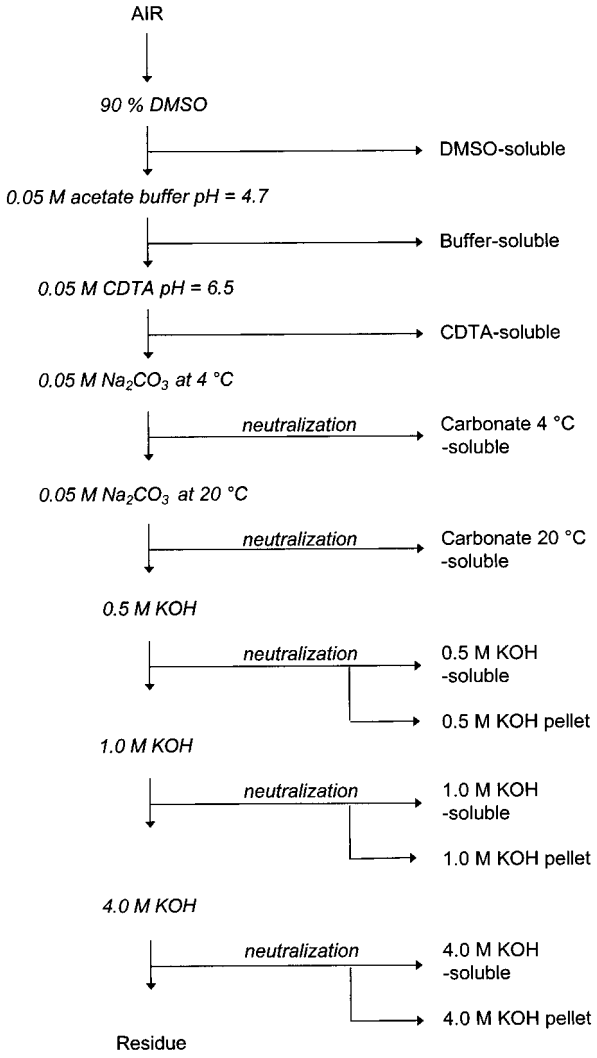


**Figure 1.** Extraction scheme for pectic and hemicellulosic cell wall fractions.

**Dry Matter Determination.** The DM content of the samples was determined by drying a known weight of homogenized sample overnight at 70 °C, followed by 3 h at 105 °C. After cooling to room temperature, the samples were weighed again. The DM content was calculated from the weight difference.

**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 according to the method described by Stolle-Smits et al. (1995).

**Isolation and Fractionation of Alcohol-Insoluble Residues and Pectic Polymers.** Fifty grams of frozen material was immersed in 180 mL of cold (−30 °C) ethanol (96% v/v), homogenized with an Ultra-Turrax T25 (Jancke und Kunkel, IKA labortechnik) by four bursts (20 500 rpm) of 1 min, and collected on a Whatman GF/C glass fiber filter. The material was suspended in 50 mL of ice-cold aqueous ethanol (80% 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. Pectic polymers were extracted using a modified method from Selvendran et al. (1985), which is summarized in Figure 1. To remove starch, the AIR (2 g) was suspended in 100 mL of 90% DMSO and stirred for 16 h at 20 °C. The suspension was centrifuged (10000*g* for 15 min) and subsequently washed twice with 90% DMSO and three times with 80% ethanol. To the pellet was added 100 mL of 0.05 M ammonium acetate buffer (pH 4.7), and the suspension was stirred for 16 h at 4 °C. The suspension was centrifuged (10000*g* for 15 min), and the pellet was washed once with the same acetate buffer and once with distilled water. To the pellet was added 100 mL of 0.05 M CDTA (pH 6.5), and the suspension was stirred for 16 h at 4 °C. The suspension was centrifuged (10000*g* for 15 min), and the pellet was washed once with the CDTA solution and once with water. To the pellet was added 100 mL of 0.05 M $Na_2CO_3$ containing 0.01 M $NaBH_4$, and the suspension was stirred for 16 h at 4 °C. At this temperature the pectin is chemically de-esterified, which renders the pectin less susceptible for $\beta$-eliminative degradation during further alkaline extractions. The suspension was centrifuged (10000*g* for 15 min). To the pellet was added 100 mL of 0.05 M $Na_2CO_3$ containing 0.01 M $NaBH_4$, and the suspension was stirred for 16 h at 20 °C. The suspension was centrifuged (10000*g* for 15 min). The depectinated residue was sequentially extracted with 0.5, 1.0, and 4.0 M KOH containing 0.01 M $NaBH_4$ by constant stirring for 16 h at 20 °C to leave a residue essentially consisting of cellulose. All extracts were filtered, neutralized, dialyzed exhaustively against demineralized water, and finally lyophilized.

**Monosaccharide Composition.** Polysaccharides from the AIR and residue were first dispersed in cold 11.5 M $H_2SO_4$ for 2 h at 20 °C. This was necessary to dissolve crystalline cellulose. Subsequently, the samples were hydrolyzed by stirring in 1 M $H_2SO_4$ for 2 h at 100 °C. The hydrolysates were filtered through a Whatman GF/C glass fiber filter and neutralized with $BaCO_3$. The pectic and hemicellulosic fractions were directly hydrolyzed by stirring in 2 M TFA for 2 h at 121 °C. Samples were dried under nitrogen at 45 °C, treated with 0.5 mL of 1 M $NH_4OH$, dried under nitrogen, and dissolved in Milli-Q water. Samples (10 $\mu$L) 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 equipped 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 an Ag/AgCl reference electrode. Pulse durations for the applied potentials were 500, 100, and 50 ms, respectively. Trehalose, added after hydrolysis of the samples, was used as an internal standard. Uronic acids were determined photometrically as described by Ahmed and Labavitch (1977). The sum of all cell wall monosaccharides was used to estimate the amount of CWM in the AIR.

**Starch Content**. To solubilize starch, 5 mL of HCl (8 M) and 20 mL of DMSO were added to 250 mg of a sample and the mixture was placed in a water bath of 60 °C. After an incubation period of 60 min under continuous shaking, 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 filtrate was used to quantify the starch content in the sample using test combination catalog no. 207748 from Boehringer Mannheim.

**Protein Content.** The nitrogen content of the AIR fractions was determined using a elemental analyzer (Carlo Erba CHNS-OEA 1108). The protein content was estimated by multiplying the nitrogen value by 6.25.

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

**Molecular Mass Determinations.** GPC was performed with a Sepharose CL-4B column (450 × 16 mm) (Pharmacia LKB). Desalted cell wall fractions (10 mg in 1.0 mL of eluens) were loaded on the column with a sample injector and were eluted with a sodium acetate buffer (0.4 M, pH 4.5) at 0.5 mL/min. Fractions of 2 mL were collected and assayed for uronic acids photometrically (Ahmed and Labavitch, 1977). The percentage of uronic acids in each fraction as a percentage of total yield of uronic acids in all fractions was calculated.

Dextran standards (Pharmacia) ranging in average molec-

**Table 1. Firmness, Yield of DW, and AIR of Green Bean Cultivars Masai and Odessa[a]**

| treatment | firmness (N) | DW (mg/g of fresh wt) | AIR (mg/g of DW) |
|---|---|---|---|
| cv. Masai | | | |
| fresh | 3754 ± 274 | 110 ± 3 | 714 ± 9 |
| blanched | 3031 ± 54 | 94 ± 1 | 761 ± 11 |
| sterilized | 161 ± 3 | 90 ± 3 | 775 ± 11 |
| cv. Odessa | | | |
| fresh | 3527 ± 106 | 105 ± 3 | 617 ± 17 |
| blanched | 2793 ± 371 | 84 ± 4 | 682 ± 17 |
| sterilized | 123 ± 13 | 89 ± 3 | 674 ± 38 |

[a] $n$= 4, ± SD.

**Table 2. Composition of the AIR of Green Bean Cultivars Masai and Odessa**

| treatment | CWM[a*] | starch (mg/g of AIR) | protein |
|---|---|---|---|
| cv. Masai | | | |
| fresh | 310 | 276 | 314 |
| blanched | 317 | 283 | 320 |
| sterilized | 291 | 278 | 390 |
| cv. Odessa | | | |
| fresh | 310 | 284 | 300 |
| blanched | 324 | 284 | 355 |
| sterilized | 282 | 310 | 308 |

[a] CWM is estimated from the total of cell wall monosaccharides in the AIR ($n$ = 2).

ular mass from 2 000 000 to 9 300 Da were applied to the column to be able to compare the size of the fractionated polymers with data from the literature. Since the cell wall polymers most likely will have a different conformation than the dextran standards, thereby affecting the elution behavior, all $M_r$ values given in this paper should be regarded as "apparent" values.

## RESULTS

**Textural Measurements.** Tissue firmness, as measured with an Instron, of both green bean cultivars decreased approximately 25-fold during processing (Table 1). The values for the fresh and blanched beans were comparable for both cultivars, but beans of cv. Masai remained firmer after sterilization than the beans of cv. Odessa.

**Isolation and Composition of AIR.** The yields of AIR were higher in the processed samples as compared with the fresh samples (Table 1). The AIR was used as the source of crude CWM. Besides CWM (28−32%), as estimated from the total amount of cell wall sugars, the AIR also contained starch (28−31%) and protein (30−39%) (Table 2).

Comparison of the crude cell wall composition of fresh beans revealed that cv. Masai beans contained relatively less arabinose and galactose and more mannose than cv. Odessa beans (Figure 2). Both the DM and the amount of acetyl constituents of both cultivars were comparable (Table 3). During sterilization the total UA content of the AIR decreased by 12 and 24% for cvs. Masai and Odessa, respectively, as can be calculated from the molar sugar composition and the yields of cell wall material (Table 2 and Figure 2). This reduction was accompanied by a slight loss of galactose. This resulted in a relative increase of other sugars in the cell walls of both cultivars. Sterilization also had a significant effect on the DM of the cell walls from both cultivars (Table 3). The DM of the AIR of sterilized beans was on average 17 percentage points lower as compared with the same samples of fresh and blanched beans. The total amount of acetyl substituents remained constant for cv. Masai beans but decreased in cv. Odessa beans (Table 3).

**Extractability and Composition of the Cell Wall Fractions.** The pectic polysaccharides not connected to other cell wall polymers were extracted with the acetate buffer; the $Ca^{2+}$-complexed polymers were most likely solubilized by CDTA. Most of the CDTA-insoluble pectins were subsequently extracted by dilute $Na_2CO_3$ at 4 and 20 °C, presumably by hydrolysis of weak ester cross-links. This residue was subsequently treated with several KOH solutions to extract hemicelluloses and some residual pectins. The CWM of the beans generally consisted of about 47−50% of pectins (total of buffer up to $Na_2CO_3$ extractions), 21−24% of hemicelluloses (total of KOH extractions), and 16−21% of cellulose (glucose in cellulose residue).

The yield of the various cell wall fractions of differently processed green beans and changes in cell wall sugar content of the various cell wall fractions are presented in Table 4. The DMSO extract also contained a large quantity of starch (data not shown) and some xylose (11−22 mol %) and mannose residues (11−29 mol %). As expected, the buffer, CDTA, and both $Na_2CO_3$ extracts were rich in pectic polysaccharides as can be deduced from their high levels of galacturonic acid residues. The most abundant neutral sugar in all pectic fractions was galactose, followed by arabinose. The ratios neutral/acid pectic sugars in the buffer, CDTA, and $Na_2CO_3$ (4 and 20 °C) from fresh beans were 0.12, 0.06, 0.13, and 0.60, respectively, for cv. Masai and 0.20, 0.15, 0.22, and 1.45, respectively, for cv. Odessa. The $Na_2CO_3$ (20 °C)-soluble fraction from all samples contained much larger amounts of neutral sugars as compared with the other pectic fractions. The pectic extracts derived from cv. Masai contained relatively less neutral sugars and more uronic acids than extracts isolated from cv. Odessa.

Hemicelluloses and additional pectins were subsequently extracted from the depectinated AIR with several KOH solutions of increasing strength. After neutralization of the KOH extracts, a pellet was formed, which was analyzed separately (Table 5). The polysaccharides in the 0.5 and 1.0 M KOH pellets were most likely primarily composed of pectins, since they contained large amounts of galacturonic acids, galactose, and arabinose. All soluble KOH extracts were also still rich in pectins, as can be deduced from the relatively high amounts of galacturonic acid, galactose, and arabinose. In addition, the 1.0 and 4.0 M KOH extracts were rich in xylose- and glucose-containing hemicelluloses.

The cell wall changes occurring during processing were comparable for both cultivars. Most importantly, a change in extractability of the pectic polymers during processing was observed. After blanching, more pectins were soluble in CDTA, although the composition of this fraction was not altered. In addition, due to the sterilization procedure, considerably more pectins were extracted with DMSO and buffer. This increase in higher levels of easily extractable material during processing was accompanied by a strong reduction of $Na_2CO_3$-extractable components. It is possible that pectins have shifted from $Na_2CO_3$ to the CDTA fractions and pectins from CDTA to the buffer and DMSO fractions. In addition, the amounts recovered in the KOH pellets were different after processing. Before processing, substantial pellet formation was observed in the 0.5 M KOH extract, while after sterilization most

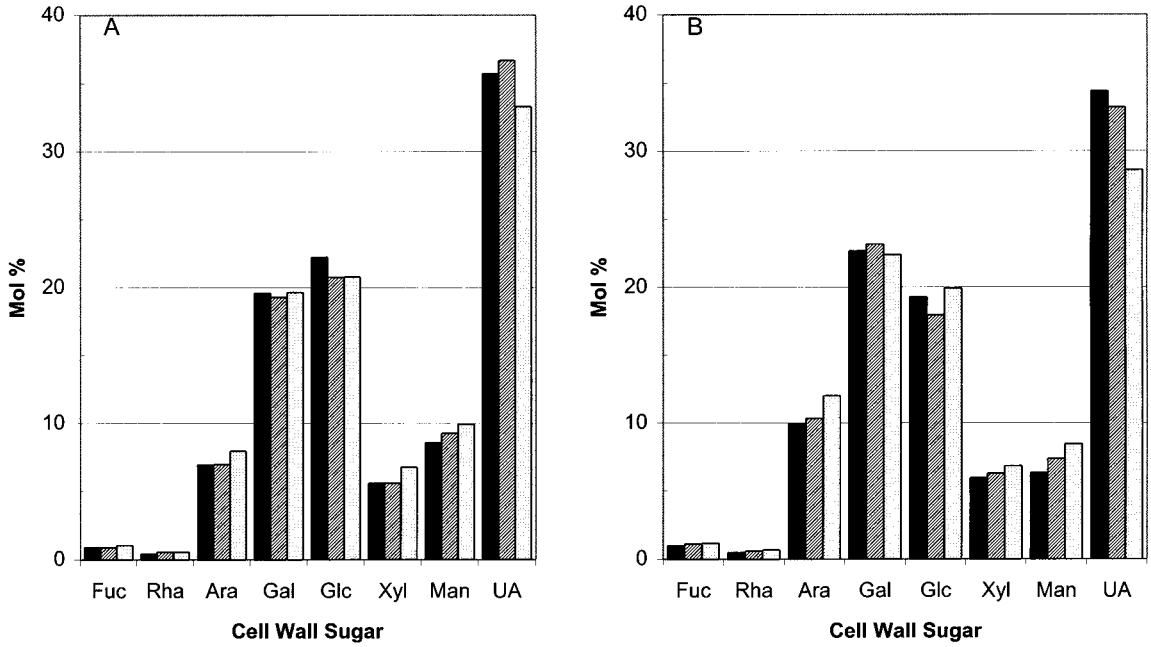


**Figure 2.** Neutral sugar composition and UA content of AIR from green bean cvs. (A) Masai and (B) Odessa: (solid bars) fresh; (striped bars) blanched; (dotted bars) sterilized.

**Table 3. DM and Number of Acetyl Groups in AIR from Green Bean Cultivars Masai and Odessa during Processing ($n = 4$)**

| treatment | DM (% of UA) | acetyl ($\mu$mol/g of AIR) |
|---|---|---|
| cv. Masai | | |
| fresh | 53.6 ± 5.0 | 235 ± 10 |
| blanched | 45.6 ± 2.5 | 247 ± 5 |
| sterilized | 35.8 ± 2.0 | 236 ± 6 |
| cv. Odessa | | |
| fresh | 50.3 + 1.0 | 240 ± 7 |
| blanched | 48.5 ± 1.9 | 256 ± 14 |
| sterilized | 34.6 ± 1.8 | 207 ± 22 |

pellet was obtained in the 1.0 and 4.0 M KOH fractions. Interestingly, sugar analyses of the pectic fractions showed that, in addition to the extractability of the pectins, also the composition of the different fractions was modified during the sterilization process. There was a general loss of galacturonic acids, resulting in a relative increase in neutral pectic sugars in all pectic extracts. Galacturonic acid and galacturonic acid oligomers were most likely lost during the cell wall isolation procedure, since they are readily soluble in 80% ethanol. The ratios neutral/acid pectic sugars in the buffer, CDTA, and $Na_2CO_3$ (4 and 20 °C) after sterilization were 0.47, 0.31, 1.07, and 2.01, respectively, for cv. Masai and 0.68, 0.41, 1.07, and 2.84, respectively, for cv. Odessa. Especially the pectins extracted with $Na_2CO_3$ showed strongly increased levels of galactose and arabinose upon sterilization. The levels of galactose increased even more than the levels of arabinose. Also, in the 1.0 M soluble KOH-fraction and all of the KOH pellet fractions the ratio between the neutral pectic sugars changed during sterilization.

**Molecular Mass Distribution ($M_r$) of the Pectic Fractions.** The $M_r$ distribution of the pectic fractions was determined by GPC (Figures 3−6). As in many other papers on plant cell wall matrix polysaccharides, our Sepharose column was calibrated using heterodisperse dextran standards (Talbott and Ray, 1992). Buffer- and CDTA-soluble material of the fresh and blanched beans contained considerable amounts of high (40−50 mL elution volume) and intermediate (50−60 mL elution volume) $M_r$ material. The pectic polymers of both $Na_2CO_3$ fractions were predominantly of intermediate $M_r$. For cv. Odessa it seemed that the 4 °C $Na_2C_3$-soluble polymers were of slightly lower $M_r$ than the cv. Masai polymers (Figure 5). For 20 °C $Na_2CO_3$-soluble polymers of cv. Odessa a relatively large peak eluted at the void volume (Figure 6), which might be caused by the presence of large molecular aggregates formed during lyophilization of the samples. Sonification or heating of the sample did not result in removal of this high $M_r$ peak. After blanching, it seemed that a small part of the polymers eluting after 50−60 mL had shifted from the buffer-soluble fraction to the CDTA-soluble fraction. This was most obvious for cv. Masai. All pectic fractions of both cultivars showed a marked reduction in $M_r$ after processing (Figures 3−6). The decrease occurred mainly during the sterilization process. The $Na_2CO_3$ fractions from sterilized green beans dissolved very difficultly and formed gels. Only very little sample material could be properly applied to the column, which resulted in a low signal to noise ratio (Figures 5 and 6).

## DISCUSSION

Our primary approach to understanding the relationship between cell wall composition and vegetable texture after processing was to compare cell walls of fresh and processed green bean cultivars that had different firmness after processing. From experience it is known that cv. Masai generally produced firmer beans than cv. Odessa; the present study confirmed this. In this paper, we focused on changes in the chemical composition of cell wall polymers during the conventional industrial preservation process.

**Composition of the Fresh Beans.** The amount of AIR, which reflects all high molecular mass components of the beans, was higher in cv. Masai as compared with cv. Odessa (Table 1). The compositions of the AIR from fresh beans, however, were comparable for both cultivars. This implies that cv. Masai contains more high molecular mass components including CWM, starch, and proteins than cv. Odessa. This effect is even more pronounced on a fresh weight basis. In addition, the overall compositions of the cell walls were slightly

**Table 4. Sugar Composition[a] and Cell Wall Yield of Pectic Fractions from Green Bean Cultivars Masai and Odessa during Industrial Processing**

| | mol % (of total cell wall sugars) | | | | | | | | | | | | | | | | cell wall yield (mg/of AIR) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fuc | | Rha | | Ara | | Gal | | Glc[b] | | Xyl | | Man | | UA | | | |
| fraction | M[c] | O[d] | M | O | M | O | M | O | M | O | M | O | M | O | M | O | M | O |
| DMSO | | | | | | | | | | | | | | | | | | |
| fresh | 8.3 | 6.7 | 0.0 | 0.0 | 8.3 | 13.3 | 15.0 | 16.7 | 0.0 | 0.0 | 21.7 | 20.0 | 28.3 | 20.0 | 18.4 | 23.2 | 47 | 34 |
| blanched | 7.2 | 5.4 | 0.0 | 0.0 | 8.6 | 12.9 | 15.8 | 19.3 | 0.0 | 0.0 | 18.6 | 19.3 | 22.9 | 22.6 | 26.9 | 20.5 | 43 | 52 |
| sterilized | 3.6 | 3.0 | 0.7 | 0.0 | 9.4 | 10.9 | 24.0 | 30.2 | 0.0 | 0.0 | 10.9 | 10.9 | 10.9 | 11.5 | 40.4 | 33.5 | 93 | 87 |
| buffer | | | | | | | | | | | | | | | | | | |
| fresh | 0.7 | 0.3 | 0.2 | 0.6 | 3.4 | 5.1 | 6.7 | 10.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.7 | 0.6 | 88.3 | 82.8 | 38 | 38 |
| blanched | 0.9 | 0.3 | 0.3 | 1.0 | 3.7 | 6.5 | 8.6 | 14.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.4 | 86.5 | 76.1 | 40 | 28 |
| sterilized | 0.2 | 0.7 | 1.6 | 1.9 | 6.1 | 8.7 | 24.3 | 29.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.4 | 67.8 | 58.2 | 72 | 57 |
| CDTA | | | | | | | | | | | | | | | | | | |
| fresh | 0.0 | 0.0 | 0.4 | 0.5 | 2.0 | 4.4 | 3.5 | 8.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 94.2 | 86.7 | 39 | 30 |
| blanched | 0.0 | 0.2 | 0.5 | 0.5 | 1.7 | 2.9 | 2.9 | 5.6 | 0.0 | 0.0 | 0.0 | 0.3 | 0.0 | 0.0 | 94.9 | 90.4 | 57 | 43 |
| sterilized | 0.0 | 0.3 | 1.3 | 1.6 | 5.8 | 8.8 | 16.5 | 19.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 76.4 | 69.7 | 40 | 38 |
| carbonate 4 °C | | | | | | | | | | | | | | | | | | |
| fresh | 0.3 | 0.4 | 0.6 | 0.8 | 2.9 | 5.2 | 7.6 | 12.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 88.6 | 81.3 | 73 | 96 |
| blanched | 0.7 | 0.6 | 1.0 | 1.4 | 3.0 | 6.0 | 7.7 | 14.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 87.6 | 77.7 | 79 | 89 |
| sterilized | 0.9 | 0.0 | 0.0 | 1.0 | 10.3 | 13.4 | 41.0 | 37.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 47.8 | 48.3 | 40 | 45 |
| carbonate 20 °C | | | | | | | | | | | | | | | | | | |
| fresh | 0.4 | 0.3 | 1.4 | 2.2 | 7.8 | 12.9 | 28.1 | 43.5 | 0.0 | 0.0 | 0.0 | 0.6 | 0.0 | 0.2 | 62.3 | 40.4 | 40 | 58 |
| blanched | 0.4 | 0.3 | 2.0 | 2.4 | 9.3 | 13.5 | 37.8 | 46.1 | 0.0 | 0.0 | 0.4 | 0.0 | 0.0 | 0.3 | 50.1 | 37.5 | 26 | 64 |
| sterilized | 2.0 | 0.0 | 2.0 | 2.4 | 15.3 | 21.8 | 48.1 | 49.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 32.5 | 26.0 | 17 | 16 |

[a] Values after TFA hydrolysis. Values are the mean of duplicate analyses, and for any value the error is <10%. [b] Glucose values are corrected for non-cell-wall glucose derived from starch. [c] Cv. Masai. [d] Cv. Odessa.

**Table 5. Sugar Composition[a] and Cell Wall Yield of Hemicellulosic Fractions and Cellulose Residue from Green Bean Cultivars Masai and Odessa during Industrial Processing**

| | mol % (of total cell wall sugars) | | | | | | | | | | | | | | | | cell wall yield (mg/g of AIR) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fuc | | Rha | | Ara | | Gal | | Glc | | Xyl | | Man | | UA | | | |
| fraction | M[b] | O[c] | M | O | M | O | M | O | M | O | M | O | M | O | M | O | M | O |
| 0.5 M KOH soluble | | | | | | | | | | | | | | | | | | |
| fresh | 0.7 | 0.6 | 1.2 | 1.4 | 16.9 | 16.3 | 52.8 | 49.6 | 0.0 | 6.7 | 11.1 | 9.3 | 0.0 | 1.5 | 17.3 | 14.6 | 18 | 29 |
| blanched | 0.0 | 0.5 | 0.8 | 1.6 | 12.8 | 14.4 | 40.8 | 42.1 | 13.3 | 17.4 | 12.9 | 8.4 | 1.3 | 0.0 | 18.1 | 15.6 | 19 | 30 |
| sterilized | 0.7 | 0.5 | 1.7 | 2.0 | 17.2 | 19.5 | 53.4 | 44.7 | 5.2 | 13.5 | 5.2 | 4.3 | 0.0 | 0.0 | 16.6 | 15.5 | 14 | 21 |
| 0.5 M KOH pellet | | | | | | | | | | | | | | | | | | |
| fresh | 0.0 | 0.0 | 0.0 | 1.4 | 12.9 | 15.5 | 48.4 | 48.9 | 3.8 | 2.8 | 0.0 | 0.9 | 0.0 | 0.0 | 34.8 | 30.5 | 24 | 31 |
| blanched | 0.0 | 0.0 | 0.0 | 0.0 | 14.0 | 15.3 | 51.8 | 45.2 | 2.8 | 5.1 | 0.0 | 0.7 | 0.0 | 0.7 | 31.5 | 33.0 | 18 | 22 |
| sterilized | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 26.9 | 13.9 | 9.6 | 44.1 | 30.7 | 0.0 | 0.0 | 0.0 | 0.0 | 41.9 | 32.8 | 1 | 1 |
| 1.0 M KOH soluble | | | | | | | | | | | | | | | | | | |
| fresh | 1.6 | 1.7 | 0.0 | 0.0 | 7.0 | 10.5 | 23.7 | 39.1 | 15.2 | 17.7 | 39.7 | 15.6 | 4.5 | 4.3 | 8.3 | 11.0 | 13 | 9 |
| blanched | 1.3 | 1.7 | 0.0 | 0.0 | 7.3 | 10.5 | 23.0 | 39.1 | 14.2 | 25.0 | 40.2 | 7.8 | 6.2 | 4.7 | 7.9 | 11.1 | 10 | 23 |
| sterilized | 0.9 | 1.0 | 0.0 | 0.9 | 13.2 | 17.9 | 39.5 | 43.7 | 10.5 | 8.7 | 20.7 | 13.5 | 2.3 | 0.0 | 12.3 | 14.3 | 21 | 30 |
| 1.0 M KOH pellet | | | | | | | | | | | | | | | | | | |
| fresh | 0.0 | 0.0 | 0.0 | 0.0 | 18.1 | 17.7 | 42.0 | 40.3 | 11.0 | 14.7 | 2.8 | 4.9 | 0.0 | 0.0 | 26.0 | 22.3 | 11 | 14 |
| blanched | 2.6 | 0.0 | 0.0 | 0.0 | 16.2 | 15.8 | 38.7 | 33.1 | 12.6 | 24.4 | 2.7 | 4.3 | 0.0 | 0.0 | 27.2 | 22.4 | 14 | 22 |
| sterilized | 2.2 | 0.0 | 0.0 | 0.0 | 12.8 | 15.2 | 28.4 | 26.7 | 11.4 | 10.2 | 3.8 | 2.0 | 0.0 | 0.0 | 41.4 | 45.9 | 33 | 25 |
| 4.0 M KOH soluble | | | | | | | | | | | | | | | | | | |
| fresh | 2.8 | 3.2 | 0.9 | 0.7 | 9.5 | 8.6 | 35.1 | 29.9 | 22.8 | 24.1 | 18.8 | 21.4 | 5.2 | 5.3 | 5.0 | 6.8 | 36 | 37 |
| blanched | 3.0 | 2.9 | 0.6 | 0.7 | 7.6 | 9.6 | 29.2 | 29.0 | 25.6 | 21.5 | 20.4 | 18.7 | 6.5 | 6.6 | 7.0 | 10.9 | 30 | 41 |
| sterilized | 2.9 | 3.5 | 0.8 | 0.6 | 8.7 | 8.1 | 31.6 | 28.3 | 24.1 | 26.9 | 20.2 | 21.2 | 5.3 | 6.5 | 6.5 | 4.9 | 37 | 36 |
| 4.0 M KOH pellet | | | | | | | | | | | | | | | | | | |
| fresh | 0.0 | 4.9 | 0.0 | 0.0 | 7.2 | 12.1 | 18.1 | 29.1 | 18.1 | 17.0 | 43.4 | 24.3 | 0.0 | 0.0 | 13.3 | 12.6 | 1 | 2 |
| blanched | 1.1 | 0.0 | 2.3 | 3.1 | 10.3 | 18.5 | 24.0 | 46.2 | 8.0 | 10.8 | 45.7 | 12.9 | 0.0 | 0.0 | 8.6 | 8.6 | 3 | 6 |
| sterilized | 1.4 | 1.7 | 4.2 | 3.4 | 15.5 | 14.4 | 43.7 | 39.7 | 29.1 | 15.2 | 46.2 | 23.7 | 0.0 | 0.0 | 6.9 | 2.0 | 15 | 21 |
| residue | | | | | | | | | | | | | | | | | | |
| fresh | 0.0 | 0.0 | 0.0 | 0.0 | 6.4 | 7.1 | 11.0 | 13.5 | 65.5 | 63.0 | 0.0 | 0.0 | 16.5 | 15.5 | 0.7 | 0.9 | 162 | 130 |
| blanched | 0.0 | 0.0 | 0.0 | 0.0 | 5.8 | 6.9 | 11.0 | 13.6 | 65.1 | 63.0 | 0.0 | 0.0 | 17.3 | 15.5 | 0.8 | 0.9 | 205 | 131 |
| sterilized | 0.0 | 0.0 | 0.0 | 0.0 | 5.3 | 3.9 | 8.8 | 7.3 | 68.7 | 70.6 | 0.0 | 3.7 | 16.7 | 14.3 | 0.5 | 0.1 | 204 | 155 |

[a] Values after TFA hydrolysis. Values are the mean of duplicate analyses, and for any value the error is <10%. [b] Cv. Masai. [c] Cv. Odessa.

different. Cv. Masai contained slightly more cellulose and fewer pectins than cv. Odessa. Also, the overall pectin compositions were different; the amounts of neutral pectic sugars (rhamnose, arabinose, and galactose) appeared higher in cv. Odessa (neutral/acid sugars = 0.96), while the amount of uronic acids appeared relatively higher in cv. Masai (neutral/acid sugars = 0.76) (Figure 2). Since a methylation analysis of CWM of green beans pointed out that a large part of the rhamnose residues was branched, it is very likely that the arabinose and galactose residues are connected to the pectin backbone as neutral side chains (Stolle-Smits et al., 1995). This suggests that pectin from beans of cv. Masai probably contained fewer or shorter side chains as compared with the pectin from beans of cv. Odessa. There was no significant difference in the amount of methyl and acetyl substituents of both cultivars.

For fresh beans of both cultivars, the DMSO-, buffer-, and CDTA-soluble fractions comprised about 3, 23, and

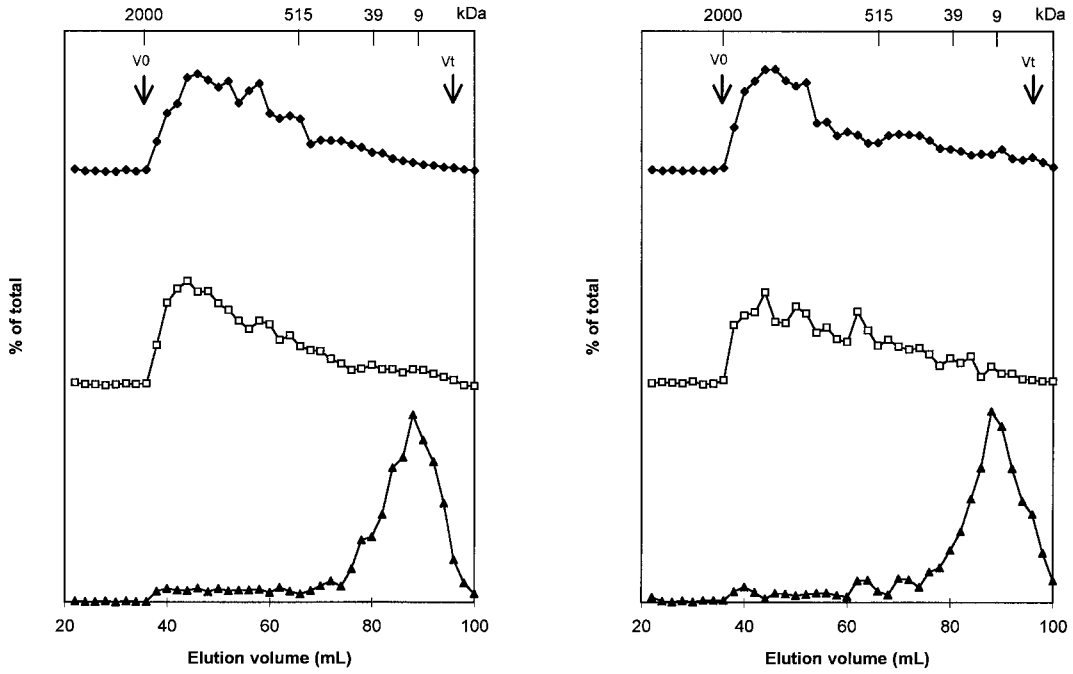


**Figure 3.** GPC profiles of buffer-soluble fractions from green bean cvs. Masai (left) and Odessa (right) after different stages of industrial processing: fresh (◆), blanched (□), and sterilized (▲). Column fractions were assayed for UA.

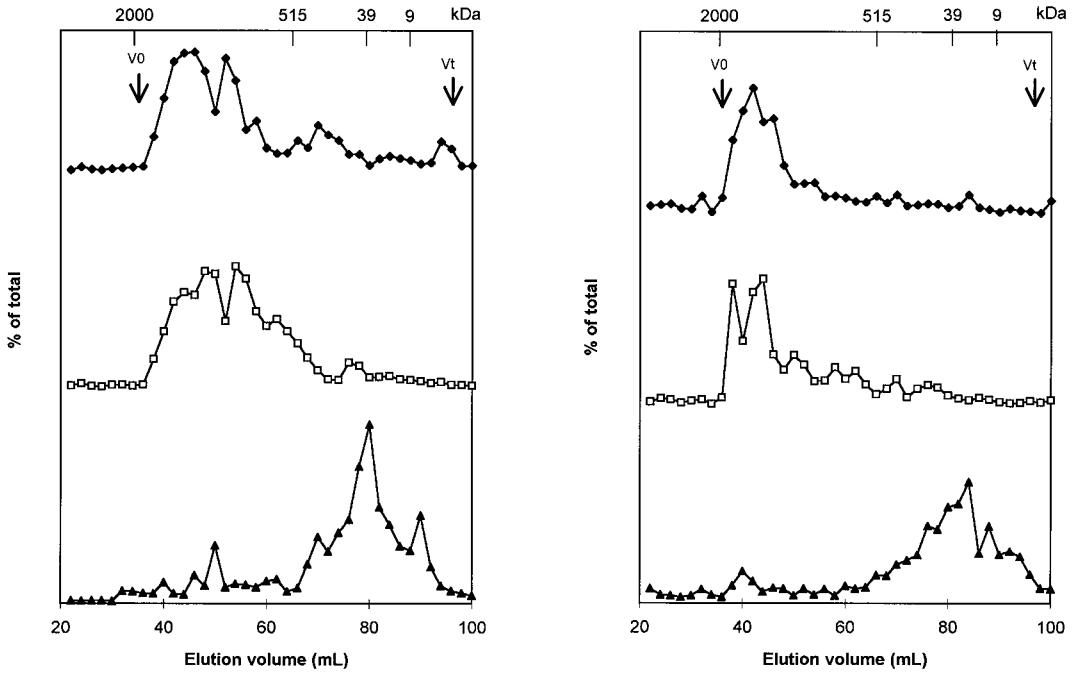


**Figure 4.** GPC profiles of CDTA-soluble fractions from green bean cvs. Masai (left) and Odessa (right) after different stages of industrial processing: fresh (◆), blanched (□), and sterilized (▲). Column fractions were assayed for UA.

24%, respectively, of the total extracted pectic substances on a uronide basis. The $Na_2CO_3$-soluble pectins made up the major fraction (47%) of the pectins from fresh beans. A small proportion (2%) of the pectins could additionally be solubilized with KOH solutions of increasing strength. Only 1% of the total uronide material remained associated with the cellulose residue after extraction. Water-soluble pectins contain free, high methoxyl pectic substances, while chelator (CDTA)-soluble pectins consist of low methoxyl pectins or salt-chelated molecules (Figure 7). Since extraction of intact potato and carrot tissues with chelating agents results in a high tendency for cell separation (Parker and Waldron, 1995), it is probable that the bulk of the CDTA-extractable pectins originate from the middle lamella. The other fractions, the $Na_2CO_3$- and hydroxide-soluble cell wall polymers, contain the insoluble and more tightly bound cell wall protopectin (Selvendran et al., 1985; Ryden and Selvendran, 1990a). $Na_2CO_3$ extractions are harsher treatments than water and CDTA extractions and are required to remove pectic substances that are probably linked to other pectins or (hemi)cellulose by weak ester bondings (Ryden and Selvendran, 1990a). Because these fractions represent the major pectic fractions of fresh green beans from both cultivars, these green beans appear to contain many such insoluble, covalently bound pectic substances. For both cultivars, the compositions of the sequential extracts were clearly different. Especially the 20 °C $Na_2CO_3$-soluble pectins appeared more branched than the

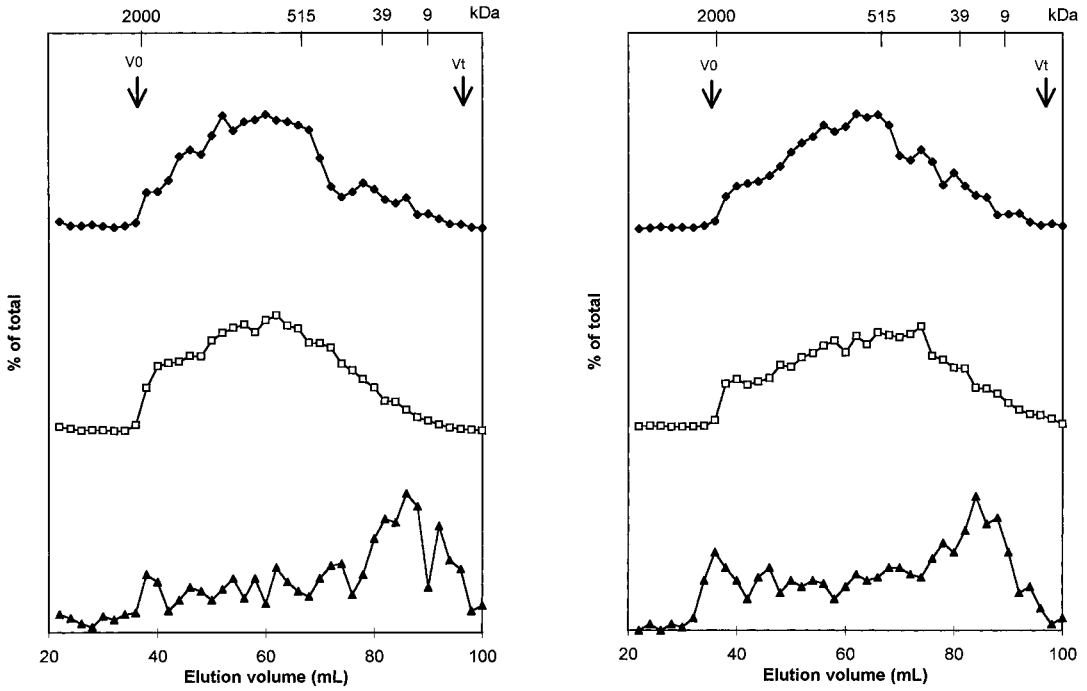


**Figure 5.** GPC profiles of carbonate (4 °C)-soluble fractions from green bean cvs. Masai (left) and Odessa (right) after different stages of industrial processing: fresh (◆), blanched (□), and sterilized (▲). Column fractions were assayed for UA.

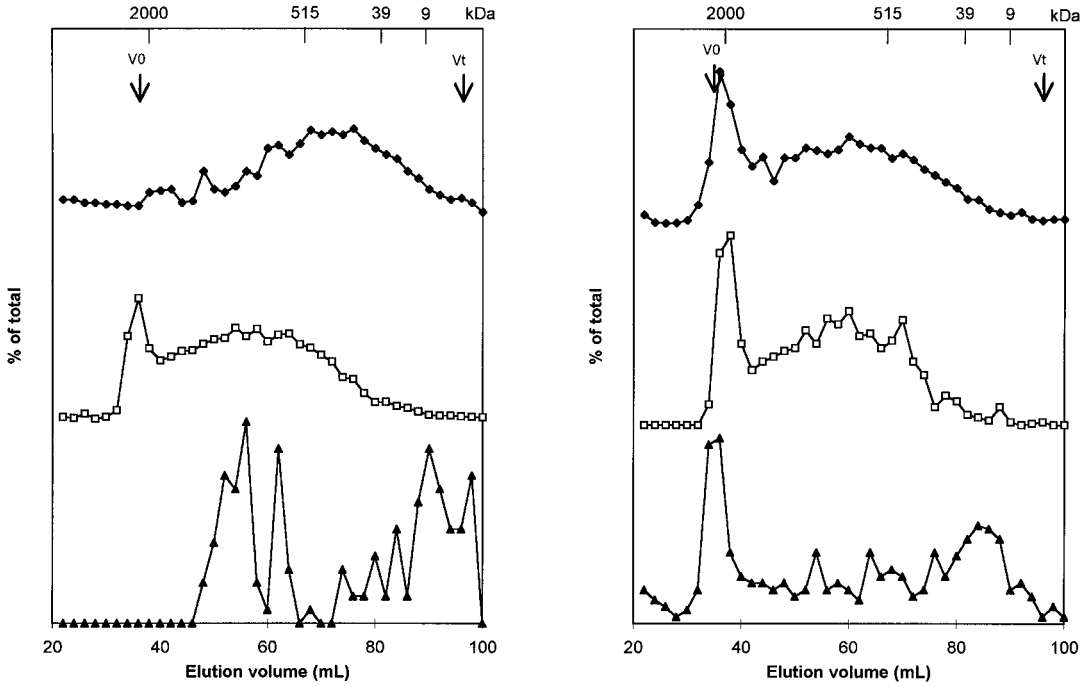


**Figure 6.** GPC profiles of carbonate (20 °C)-soluble fractions from green bean cvs. Masai (left) and Odessa (right) after different stages of industrial processing: fresh (◆), blanched (□), and sterilized (▲). Column fractions were assayed for UA.

buffer- and CDTA-extractable pectins, as can be concluded from the high rhamnose-to-galacturonic acid ratio. Rhamnose present in the pectic backbone is a major site of side-chain attachment. In fresh and blanched beans of cv. Masai there seemed to be relatively more buffer- and CDTA-extractable pectin, whereas in cv. Odessa there was more $Na_2CO_3$-extractable material. Also, the compositions of the fractions from both cultivars were different. In general, the fractions from cv. Masai contained fewer neutral pectic sugars and more galacturonic acids. This once more indicates that the pectins from cv. Masai probably contain fewer and/or shorter side chains as compared with the pectin of cv. Odessa. Previously it has been shown for apples, kiwi, and nectarines that the levels of galactose decreased during fruit ripening (Dawson et al., 1992; Redgwell et al., 1992; Fischer et al., 1994). 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. The presence of side chains in carbohydrate polymers significantly affects a variety of functional properties (Hwang et al., 1993). However, relative to other functional properties of carbohydrate polymers, like retrogradation and gelatinization of starch, the contribution of side chains to rheological properties has not been studied in depth.

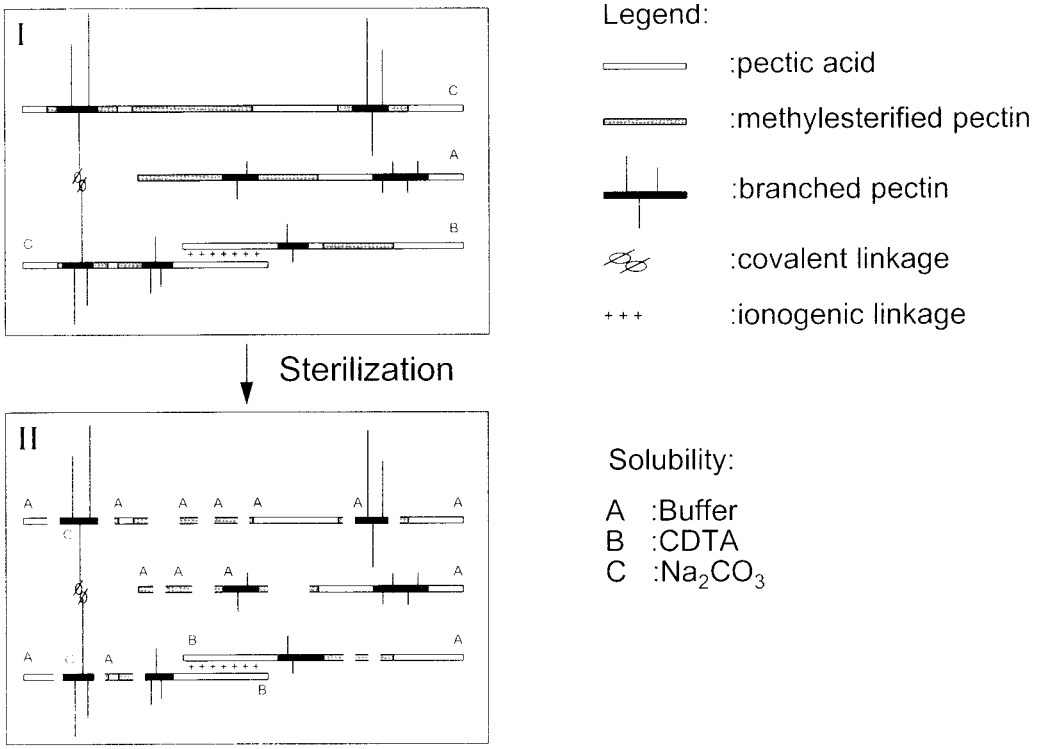


**Figure 7.** Schematic picture of suggested changes in pectic polymers during industrial processing of green beans. In the fresh beans (I) a large part of the pectin appears covalently linked to other cell wall components. During processing the pectin is partly degraded and depolymerized by $\beta$-eliminative degradation of methylated pectic regions. This results in more buffer-soluble and fewer carbonate-soluble pectins in the cell wall of sterilized green beans (II).

Two contradictory arguments have been issued for the role of side chains of pectins in gelling. Selvendran et al. (1985) stated that arabinose and galactose side chains of pectin could positively contribute to gelling by keeping water molecules within the gel framework. In contrast, BeMiller (1986) and Reid (1983) stated that the side chains of pectins might tend to limit the extent of interchain association and, thus, the formation of junction zones for gelling may be inhibited. More systematic research is needed to elucidate the role of side chains of pectins in food systems.

KOH solubilized several hemicelluloses and also some remaining pectic material. All KOH fractions contained mixtures of pectins and hemicelluloses. From these experiments, it is, however, not possible to determine whether these different polymers are covalently linked or just coextracted as separate polymers. A substantial part of the hydroxide-soluble pectic material was recovered in pellets which formed after neutralization of the fractions. This can be a result of protein−pectin complexes formed at a pH below the isoelectric point of the protein, at which the protein is cationic and the pectin is anionic (Dalev and Simeonova, 1996). Preliminary experiments indeed indicated that the pellets contained appreciable amounts of protein (data not shown). From the molar ratios of the various cell wall sugars it can be deduced that the KOH-soluble hemicellulosic polymers consisted mainly of xyloglucans. These xyloglucans differed presumably in their affinity for cellulose microfibrils, since they apparently needed different concentrations of KOH for solubilization. In contrast to cv. Odessa beans, the 1.0 M KOH extract and the 4.0 M KOH pellet of cv. Masai contained relatively large amounts of xylose. The xylose/glucose ratios for the 1.0 and 4.0 M KOH pellet were 2.61 and 2.40, respectively. Using methylation analysis of the CWM from green beans (Stolle-Smits et al., 1995), it was obvious that a large part of the xylose in green beans was (1−4)- and (1,2,4)-linked. Together with the relative amounts of xylose and glucose, this suggests that the xylose in the 1.0 M KOH extract and 4.0 M KOH pellet from especially cv. Masai is derived from xylans. Whether arabinoxylans or glucuronoxylans are present cannot be deduced from the present data. Another possibility is the presence of pectic xylogalacturonans. In the CWM of runner beans it was shown that some galacturonic acid residues were substituted with xylose on positions 2 and 3 (Ryden and Selvendran, 1990a). In addition, Ryden and Selvendran (1990a,b) stated that they found complexes composed of (1−4)-linked-xylose-containing polymers associated with pectic material in hydroxide fractions from runner beans and potatoes. They suggested that such complexes may serve as cross-linking polymers within the cell wall matrix of leguminous species. Probably cell walls of cv. Masai are more heavily cross-linked by this kind of complex than cell walls of cv. Odessa. In addition, Northcote et al. (1989) detected (1−4)-linked-xylose-containing polysaccharides by immunochemistry in the middle lamella of suspension-cultured bean cells. Waldron and Selvendran (1992) suggested that these xylan−pectic polysaccharide complexes might be associated with the concomitant deposition of lignin. Lignification is known to start from the middle lamella region and involves the deposition of phenolics with xylan. The obtained results therefore might suggest that for cv. Masai the carbohydrate initials for lignification may be present at a larger amount and that the middle lamella of this cultivar may even already contain some lignin deposits.

**Cell Wall Changes during Processing.** During sterilization pectins were solubilized (Table 4). The overall DM decreased during sterilization, probably due to complete degradation of highly methylated regions of pectin by $\beta$-elimination (Table 3). Analysis of the isolated pectic fractions of the bean pods revealed that

substantially more pectin became DMSO- and buffer-soluble after sterilization and that the $M_r$ of pectin was reduced. Pectin shifted from the $Na_2CO_3$ fractions to the DMSO, buffer, and CDTA fractions (Figure 7). During blanching, there was an increase in CDTA-extractable material. This might be an effect of some pectin methylesterase (PME) activity during the blanching treatment. Although at 90 °C PME will be inactivated very rapidly, it will take some time before the entire bean pod reaches this temperature. During this warming period PME could have demethylated some of the pectin and made these demethylated pectins available for calcium cross-linking. This idea is supported by a slight decrease of the overall DM of the pectin after blanching. After sterilization, the amount of pectins extracted with CDTA was equal to the amount extracted with CDTA from the fresh material. This is due to an overall transfer of pectic material from the different fractions. After sterilization, the $M_r$ of all fractions was strongly reduced. In addition, there was an increase of arabinose and galactose in all fractions, indicating that all fractions contained more branched pectins. This is consistent with the general idea that during sterilization linear, nonbranched, high methoxyl pectin is degraded into small fragments by $\beta$-elimination (Stolle-Smits et al., 1995). Just a very small amount of the pectins remained extractable only with alkaline solutions or associated with the cellulose residue. On the basis of their high arabinose and galactose contents, these pectins were most probably very highly branched. No important changes were observed in hemicellulosic and cellulosic polymers. The changes occurring in the hemicellulosic fractions during processing were predominantly caused by pectic polymer modifications. The major changes were changes in the yields of the different fractions, especially the pellet fractions.

In conclusion, two major effects of blanching and sterilization on the cell walls of green beans can be discriminated (Figure 7): (1) a degradation of linear regions (homogalacturonan) into monomers and small oligomers and (2) a solubilization of branched regions (rhamnogalacturonan) of the cell wall pectin. Several differences were observed between cell wall materials from beans of cvs. Masai and Odessa. It is not possible to determine whether these represent intrinsic differences, since only one batch of each cultivar was analyzed. Cultivar Masai beans, which had the highest firmness retention after sterilization, appeared to have (1) more CWM, (2) fewer branched pectins, and (3) more xylans. The present study cannot discriminate between these characteristics with regard to firmness retention because only two cultivars were studied. To determine which of these differences is most important in firmness retention, cell wall characteristics of more cultivars have to be compared.

## ABBREVIATIONS USED

AIR, alcohol-insoluble residue; Ara, arabinose; CDTA, cyclohexane-*trans*-1,2-diaminetetraacetate; CWM, cell wall material; DM, degree of methylation; DMSO, dimethyl sulfoxide; DW, dry weight; Fuc, fucose; Gal, galactose; Glc, glucose; GPC, gel permeation chromatography; Man, mannose; $M_r$, molecular mass relative to dextran standards; PME, pectin methylesterase; Rha, rhamnose; TFA, trifluoroacetic acid; UA, uronic acid; Xyl, Xylose.

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