Preheating Effects on the Textural Strength of Canned Green Beans. 1. Cell Wall Chemistry

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Variable preheating conditions allowed the modification of the firmness of two green bean cultivars after processing. The aim of this study was to elucidate the biochemical basis of this phenomenon and to relate pectin differences to different inherent firmness of two cultivars. The preheating temperature, which resulted in the highest retention of firmness after sterilization, corresponded with the optimal temperature for pectin methylesterase activity. After this preheating treatment, there was an overall reduction of the degree of methylation of the cell wall pectin. In addition, the yields of the buffer and chelator soluble fractions, as well as their average molecular mass, were higher after sterilization. Firmness differences between the two cultivars seemed to be related to the degree of methylation, the degree of acetylation, and the total amount of pectins. Preheating of green beans affects texture after sterilization most likely by demethylation of pectin by pectin methylesterase thereby (i) decreasing the β-eliminative degradation of pectin and (ii) increasing the capacity of pectin to form Ca^{2+}-mediated complexes.

Keywords: *Cell walls; pectin; food processing; preheating; Phaseolus vulgaris; pectin methylesterase; peroxidase; texture; firmness*

INTRODUCTION

Texture is an important quality attribute of fruits and vegetables. Loss of texture during industrial processing of these products is often dramatic and has been the subject of much research. At the practical level, research has been directed toward modifying processing conditions so that more of the products' original texture can be retained. At the fundamental level, research has been directed toward understanding which chemical and/or structural parameters of plant tissues contribute to texture and how the preservation process affects these features.

During heating, turgor and membrane integrity are quickly lost (Greve et al., 1994). This initial softening process is enhanced by dissolution of the cell wall and middle lamella (Greve et al., 1994; Nyman et al., 1994; Stolle-Smits et al., 1995). Mainly, the pectic polymers of the cell wall and middle lamella change during processing. In fresh beans, the major part of the pectins is most likely covalently linked to other cell wall polymers (Stolle-Smits et al., 1997). Heating resulted in degradation and solubilization of pectic polymers from the cell wall and middle lamella (Greve et al., 1994; Stolle-Smits et al., 1995, 1997; Lee et al., 1979; Plat et al., 1988). Model experiments with solubilized carrot pectins indicated that β-elimination was primarily responsible for heat degradation of pectins and that higher methyl ester content resulted in greater rates of degradation (Sajjaanantakul et al., 1989). However, little experimental data on the degradation mechanism of native plant pectin are available. The firmness loss of green beans after sterilization can be decreased by preheating the beans at moderate temperatures (Chang et al., 1993; Van Buren et al., 1960). This firming effect is generally attributed to the action of pectin methylesterase (PME) during the preheating period (Van Buren et al., 1979; Bartolome and Hoff, 1972; Chang et al., 1996). It has been hypothesized that PME forms stretches of consecutive acidic GalA residues, which will bind in a consorted way to a number of Ca^{2+} ions (Mort et al., 1993). Consequently, strong cross-links can be formed between pectin molecules, probably in a well-defined arrangement known as the "egg-box" model (Morris et al., 1982). These complexes are thought to have a firming effect on the tissue. At the moment, however, it is still not proven whether these egg-box structures really exist in vivo (Liners and Van Cutsem, 1992). Another possible firming effect during preheating may be the result of peroxidase (POD) action. A small percentage of the sugars in wall polysaccharides carry ferulic acid and related phenolic groups, which may be cross-linked by the action of POD and H_2O_2 (Fry, 1988). These cross-linked structures form intercellular bridges, such as diferulate and isodityrosine, thus connecting polymers together in a tight network. In bamboo shoots, sugar beet, and Chinese water chestnut these ferulic acid dimers have been associated with thermal stability of texture (Parker and Waldron, 1995). These phenolic dimers require a higher pH for their de-esterification than Na_2CO_3-saponifiable uronyl esters (Fry, 1986).

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Table 1. Overview of Processing Conditions of Green Bean Cultivars Masai and Odessa

sample	preheating temp (°C)	preheating time (min)	blanching 4 min, 90 °C	sterilization 30 min, 118 °C
1	30	120	yes	yes
2	40	90	yes	yes
3	50	60	yes	yes
4	60	45	yes	yes
5	70	20	yes	yes
6	80	10	yes	yes
7	90	4	no	yes

In conclusion, the details of the chemical cell wall changes at moderate preheating temperatures that cause the retention of firmness are not yet fully understood. In this paper, we relate biochemical and chemical aspects of preheating to a reduced loss of firmness during sterilization of green beans.

MATERIALS AND METHODS

Plant Material. Green beans (*P. vulgaris* L.) cv. Masai and Odessa were grown at the experimental research station PAGV (Lelystad, The Netherlands) and harvested at edible maturity. Both ends of the pods were removed, and the middle parts were cut into parts of 3–4 cm long.

Processing Conditions. Beans were processed according to Table 1. The green bean samples were placed in tap water of various temperatures (30–80 °C) and preheated for a certain period of time (10–120 min). Subsequently, the beans were blanched for 4 min at 90 °C. After the first preheating treatment, samples were frozen and lyophilized for enzyme activity measurements and cell wall analyses. For the succeeding canning process, portions (410 g) of beans were packed into glass jars (720 mL) and a 0.25 M NaCl solution (brine) was added. Closed jars were sterilized at 118 °C for 30 min. After sterilization, the samples were stored at 15 °C until firmness evaluation and cell wall analyses.

Texture Assessment. The firmness of the beans was measured using a Universal Testing Machine (Instron, High Wycombe UK) 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) needed to break through the beans was used to quantify the instrumental firmness of the beans.

Isolation of Alcohol-Insoluble Residue (AIR) and Pectic Polymers. AIR was isolated from lyophilized blanched and sterilized bean samples. Sterilized green beans (50 g) were homogenized in an equal weight volume of water; the lyophilized blanched bean samples were ground in a mill with a sieve of 0.5-mm mesh. AIR was isolated by refluxing for 30 min in 150 mL of hot ethanol (80% v/v), filtering, and finally washing the residue with 100% acetone.

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 the pellet was washed twice with 90% DMSO and three times with 80% ethanol. To the pellet, 100 mL of 0.05 M ammonium acetate buffer (pH = 4.7) was added, and the suspension was incubated under constant stirring for 16 h at 4 °C. After centrifugation (10000*g* for 15 min) was performed, the pellet was washed once with acetate buffer and once with distilled water. To the pellet, 100 mL of 0.05 M CDTA (pH = 6.5) was added, and the suspension was incubated with constant stirring 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, 100 mL of 0.05 M Na_2CO_3 containing 0.01 M $NaBH_4$ was added, and the suspension was incubated with constant stirring for 16 h at 4 °C. The suspension was centrifuged (10000*g* for 15 min). To the pellet 100 mL, of 0.05 M Na_2CO_3 containing 0.01 M $NaBH_4$ was added, and the suspension was incubated with constant stirring for 16 h at 20 °C. The suspension was centrifuged (10000*g* for 15 min).

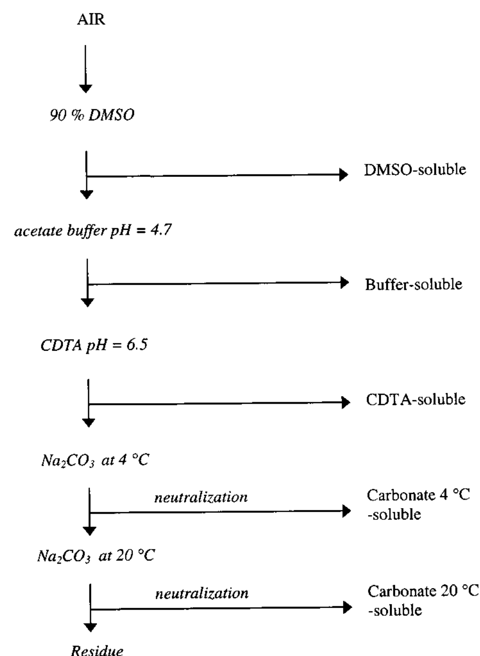


Figure 1. Extraction scheme for the pectic cell wall fractions.

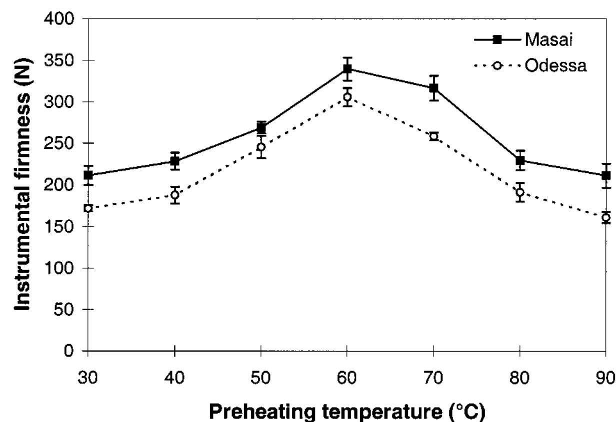


Figure 2. Firmness of sterilized green bean cultivars Masai and Odessa after different preheating treatments.

Dry Matter Determination. The dry matter content of the processed bean samples was determined as described in Stolle-Smits et al. (1997).

Monosaccharide Composition. Cell wall sugars in the AIR, residue, and solubilized pectic fractions were released and analyzed as was described in Stolle-Smits et al. (1997). Anhydro-uronic acids were determined as described by Ahmed and Labavitch (1977).

Starch Content. Starch was determined using a test-combination catalog no. 207748 from Boehringer Mannheim, Mannheim, Germany as described previously (Stolle-Smits et al., 1995).

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

Molecular Mass Distribution Analyses. High performance size exclusion chromatography (HPSEC) was performed using an HPLC system (Waters, Milford, MA, UK6 injector, Waters 510 HPLC pump) equipped with two columns (each 7.8 × 300 mm) in series (Ultrahydrogel 500 and Ultrahydrogel 250; Waters) in combination with a Waters Ultrahydrogel guard column. The samples were eluted with 0.4 M acetic acid/

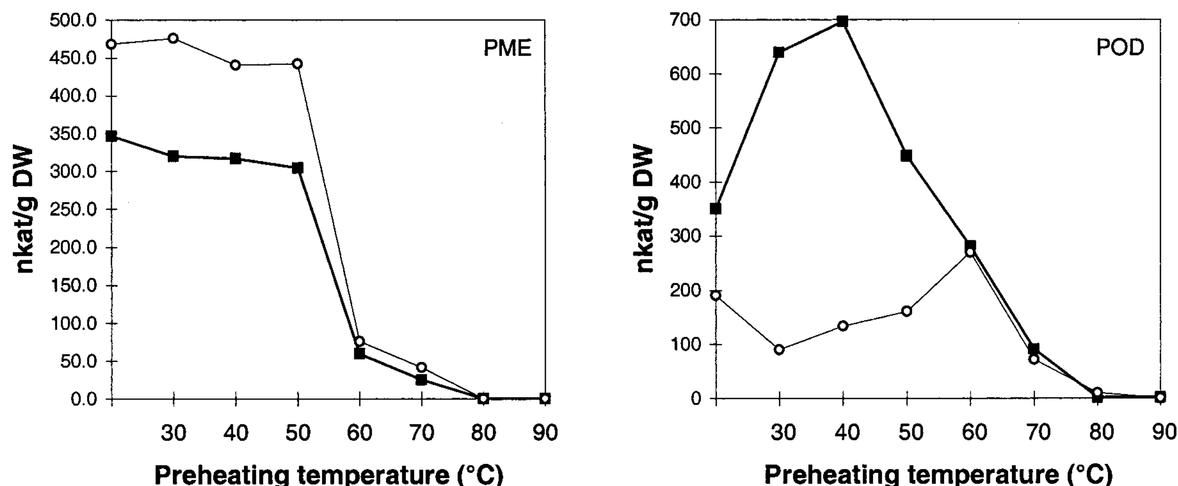


Figure 3. Residual PME and POD activity of green bean cultivars Masai (-■-) and Odessa (-○-) after preheating at different temperatures (for preheating conditions, see Table 1).

Table 2. Yield of AIR and Rhamnose, Arabinose, Galactose and Uronic Acid Content, Degree of Methylation, and Number of Acetyl Groups in AIR Isolated from Green Beans during Processing

sample	preheating temp (°C)	AIR (mg/g of DW)	pectic sugar composition (μmol/g of DW) Rha	Ara	Gal	AUA	DM (mol %)	acetyl (μmol/g of DW)
			cv. Masai					
fresh		688	31	127	193	420	65.9	161
preheated	30	727	30	125	175	421	65.1	157
	40	714	30	128	199	444	64.1	165
	50	691	30	124	211	457	58.7	160
	60	692	32	124	219	461	53.9	173
	70	681	31	131	242	446	55.8	172
	80	685	28	128	232	459	58.8	162
	90	736	30	131	206	437	67.6	172
preheated + sterilized	30	745	32	152	249	356	48.5	174
	40	761	29	144	230	357	49.0	172
	50	762	33	152	245	360	44.6	170
	60	767	37	171	284	401	41.8	189
	70	763	34	150	251	420	37.7	177
	80	755	34	143	248	396	45.1	176
	90	742	35	137	218	277	55.9	169
			cv. Odessa					
fresh		661	21	98	156	320	68.7	121
preheated	30	698	18	99	164	335	71.2	133
	40	591	17	93	156	322	64.1	123
	50	709	19	98	150	346	65.5	142
	60	726	21	103	157	372	55.5	153
	70	739	21	114	180	377	53.6	144
	80	659	24	103	192	341	63.5	139
	90	650	19	101	204	346	67.3	140
preheated + sterilized	30	716	28	114	191	265	55.5	136
	40	724	27	115	198	258	51.8	134
	50	732	24	130	208	281	48.3	134
	60	730	28	135	229	310	47.9	147
	70	731	31	122	205	297	45.9	137
	80	716	28	122	217	301	45.4	138
	90	730	22	118	182	261	48.9	121

sodium acetate (pH = 3.0). The eluate was monitored using a refractive index detector (Pharmacia LKB, Uppsala). The system was calibrated using linear pullulans with molecular masses in the range of 6000−1 660 000 Da. Data analysis was performed using Millennium 2000 software (Waters).

Enzyme Activity Measurements. *PME Extraction and Activity Assay.* Ground, lyophilized bean powder (40 mg) was first washed for 1 h at 4 °C with 1.0 mL demineralized water and subsequently extracted with 1.0 mL 1 M NaCl for 1 h at 4 °C. After centrifugation, the total PME activity in the supernatant was determined using a continuous spectrophotometric assay with bromothymol blue as a pH indicator (Hagerman and Austin, 1986). The reaction mixture (3000 μL) contained 0.14% citrus pectin (Sigma-Aldrich Chemie, Zwijndrecht, NL), 0.028% bromothymol blue, and 100 mM NaCl, pH = 7.8. The reaction was started by adding 100 μL of sample solution, and the decrease in absorbancy at 616 nm was monitored using an UVIKON spectrophotometer. PME activities were determined using galacturonic acid as a standard and expressed in katals (1 katal = 1 mole product formed/s).

POD Extraction and Activity Assay. Ground, lyophilized bean powder (100 mg) was extracted with 1.0 mL of 0.1 M phosphate buffer, pH = 6.0, for 1 h at 4 °C. After centrifugation was peformed, the activity of soluble POD in the supernatant was determined using a continuous spectrophotometric assay. The reaction mixture (1900 μL) consisted of 0.16 mM dianisidine and 13 mM H_2O_2, pH = 6.0. The reaction was started by adding 300 μL of sample solution, and the increase in absorbancy at 460 nm was monitored using an UVIKON spectrophotometer. POD activities were determined using the

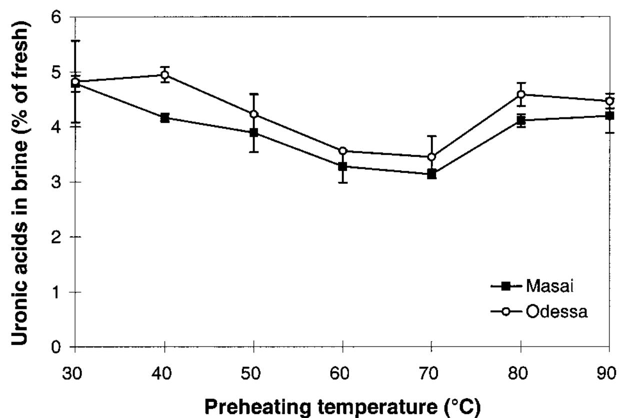


Figure 4. Uronic acids leached out into the brine during sterilization of two cultivars of green beans after different preheating conditions. Values are expressed as percentage of total uronic acids present in fresh green beans (for preheating conditions, see Table 1) ($n = 2$).

molar extinction coefficient of dianisidine (11.3×10^{-6}) and expressed in katals (1 katal = 1 mole product formed/s).

Protein Content. Protein in the fractions was analyzed with the Coomassie Plus Protein Assay Reagent from Pierce (catalog no. 23236) using BSA as reference protein.

RESULTS

Firmness Measurements. Tissue firmness of the sterilized green beans was determined using an Instron Universal Testing machine equipped with a Kramer shear cell. Beans of cv. Masai were firmer than beans of cv. Odessa after any processing condition. Maximal firmness values after sterilization were obtained for the beans of both cultivars after preheating at 60 °C (Figure 2).

Enzyme Activity Measurements. Bean PME and POD residual activities were compared after different preheating treatments (Figure 3). PME and POD activities were higher in cv. Masai as compared with cv. Odessa. The PME activity was not altered by preheating at 30, 40, and 50 °C. Some PME activity was still detected after preheating at 60 and 70 °C, but after preheating at 80 or 90 °C, no activity was found. The activity of soluble POD from cv. Masai was strongly increased by preheating at 30 or 40 °C. For cv. Odessa, we could not detect a significant increase of POD activity after preheating. The POD activity decreased by heating at 70 or 80 °C. After blanching at 90 °C, no POD activity was detectable anymore.

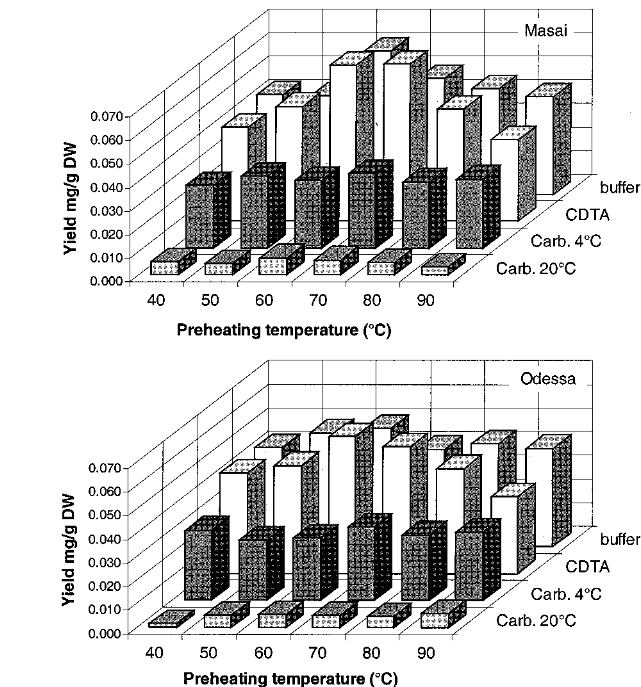
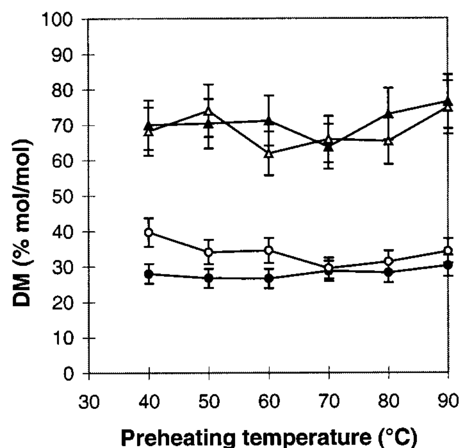


Figure 5. Yields of sequential pectic fractions isolated from green bean cultivars Masai and Odessa after sterilisation and different preheating treatments (for preheating conditions, see Table 1).

Analysis of Alcohol-Insoluble Residues. The pectic sugar composition of all AIR samples was compared (Table 2). Interestingly, beans of cv. Masai seemed to contain more pectic material than beans of cv. Odessa. There was no significant difference in the pectin composition of the bean samples of the two cultivars after different preheating treatments. Uronic acid was lost from the AIR during sterilization. After sterilization, pectin was the least degraded in the beans preheated at 60 °C.

Analysis of the degree of methylation (DM) and acetyl esters in the AIR samples from preheated beans revealed that the DM was lowered in beans blanched at 50, 60, 70, and 80 °C in comparison with fresh beans (Table 2). The amount of acetyl esters remained constant during all preheating treatments. Analysis of the



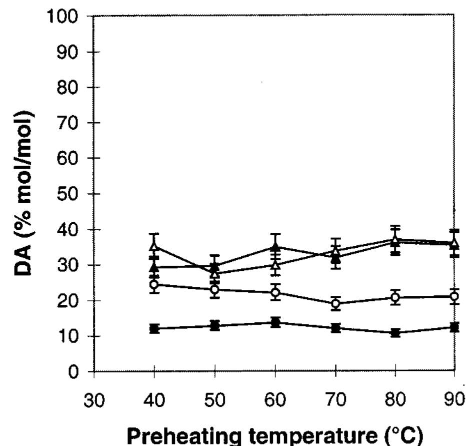


Figure 6. Degree of methylation (DM) and acetylation (DA) of buffer (triangles) and CDTA (circles) soluble pectic fractions isolated from green bean cultivars Masai (solid markers) and Odessa (open markers) after sterilization and different preheating treatments. (for preheating conditions see Table 1).

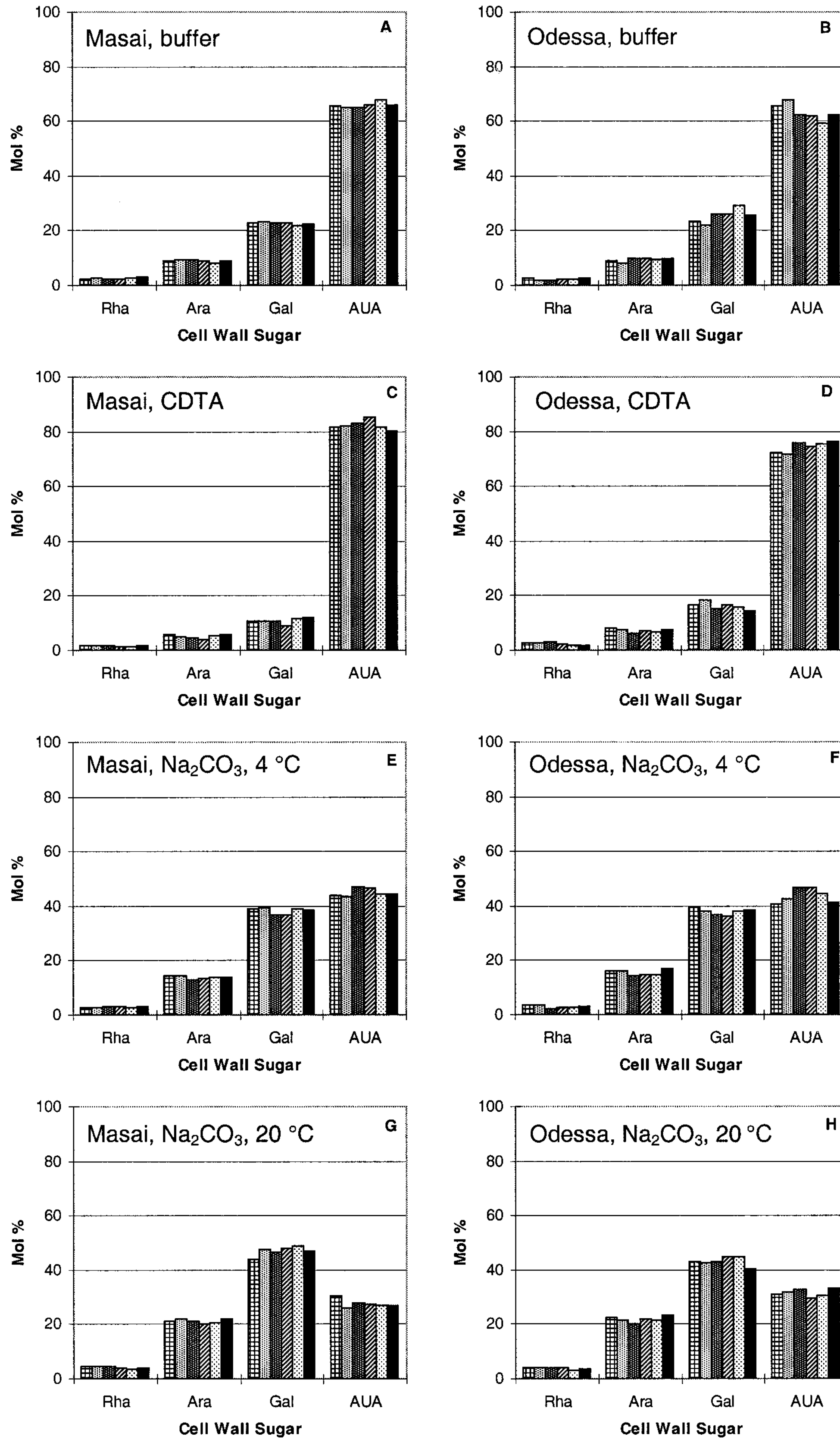


Figure 7. Composition of sequential pectic fractions isolated from green bean cvs. Masai and Odessa after sterilization and different preheating treatments (white bars with black lines, 40 °C, 120 min; white bars with dense black dots, 50 °C, 90 min; black bars with white dots, 60 °C, 45 min; white bars with diagonal black lines, 70 °C, 20 min; white bars with thin white dots, 80 °C, 10 min; solid black bars, 90 °C, 4 min) (for preheating conditions see Table 1).

DM of the sterilized beans showed that for both cultivars the DM was still slightly lower in the 60−80 °C preheated samples. The difference was, however, much lower than in the only preheated samples.

Solubility and Composition of Cell Wall Pectins. The uronic acids solubilized into the brine during

sterilization were quantified, revealing that there was less pectin degradation in the bean samples preheated at 50−80 °C (Figure 4). Relatively more pectin was degraded in green beans of cv. Odessa than in beans of cv. Masai for all processing conditions.

The AIR from the preheated (40−90 °C) samples was fractionated according to Figure 1. This extraction procedure was designed to minimize β-eliminative degradation of pectins during the initial stages of extraction and to solubilize the polymers in as close to their native form as possible (Selvendran et al., 1985). The pectic polysaccharides not connected to other cell wall polymers were extracted with acetate buffer; the polymers bound in the wall by Ca^{2+} only were 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 (Fry, 1986). Cold Na_2CO_3, which would hydrolyze ester bonds, but cause negligible elimination−degradation, solubilized most of the CDTA-insoluble pectins. The sugar composition, DM, number of acetyl substituents, and molecular mass distribution of the resulting pectic fractions and residue was compared. The overall yield of the different fractions is visualized in Figure 5. Substantially more pectins could be extracted from beans preheated at 60 or 70 °C. This effect was most obvious in cv. Masai. Essentially, the amounts of buffer and CDTA-soluble material increased at these temperatures. The degree of methylation and acetylation of the buffer and CDTA fractions showed no clear trend in relation to the different preheating treatments (Figure 6). For most samples, both the degree of acetylation and methylation of the CDTA-soluble pectins from cv. Odessa were higher in comparison with the CDTA soluble pectins from cv. Masai. For all pectic fractions, the pectin yield was higher in the samples preheated at moderate temperatures. In addition, the amount of pectin as expressed per gram dry weight of beans was higher for cv. Masai as compared with cv. Odessa. However, the sugar composition of all fractions was the same for almost all samples (Figure 7). The CDTA-soluble polymers from cv. Odessa contained relatively more neutral pectic sugars than the CDTA-soluble pectins from cv. Masai suggesting that they contain more or longer side chains. In the 4 °C carbonate extracts, a trend was observed toward a slightly higher relative uronic acid content in the samples preheated at 60 or 70 °C. There was no effect of preheating on the yield and composition of the residue of the sterilized beans (Table 3). A relative high proportion of the uronic acid containing polymers remained associated with the "depectinated" residue.

Molecular Mass Analyses. The molecular mass (M_r) distribution of all pectic fractions was determined by HPSEC as shown in Figure 8. For each fraction, the differently preheated samples had fairly similar elution patterns, and only the relative amounts of the peaks varied. The buffer and carbonate soluble polymers showed three major peaks, eluting at 13, 14, and 20 min, respectively. The molecular mass distribution of the buffer and 4 °C carbonate soluble fractions of beans preheated at 50−70 °C showed a higher peak eluting at 14−15 min, in comparison to the conventionally (90 °C) blanched sample. This was most obvious for cv. Odessa. The CDTA-soluble polymers showed a large peak eluting at 17−18 min. The CDTA-soluble pectins of beans preheated at 60 or 70 °C were of higher molecular mass than pectins from the other samples. Also, a small peak eluting at 19−20 min became visible in the chromatograms. In these fractions, only small amounts of high molecular material, eluting at 13−14 min, was found. Preheating at 50−60 °C resulted in just slight alterations of the elution patterns of the 20 °C carbonate-soluble polymers.

Table 3. Yield and Composition of Residue after Extraction of Pectins from the AIR of Green Bean Cultivars Masai and Odessa (*n* = 2)

preheat temp (°C)	res yield (mg/g DW)	cell wall sugars in residue (mol %)							
		Fuc	Rha	Ara	Gal	Glc	Xyl	Man	AUA
			cv. Masai						
40	0.428	0.3	nd[a]	4.9	5.4	59.1	3.9	3.9	22.5
50	0.438	0.4	nd	4.7	5.5	60.6	3.9	3.6	21.3
60	0.404	0.5	nd	4.6	6.0	59.5	4.0	3.9	21.5
70	0.427	0.5	nd	5.0	5.9	58.3	4.2	3.9	22.2
80	0.421	0.5	nd	4.9	6.4	61.7	4.1	3.9	18.5
90	0.417	0.5	nd	4.8.	5.5	61.6	4.0	3.8	19.8
			cv. Odessa						
40	0.394	0.5	nd	4.0	5.3	64.0	3.3	3.2	19.6
50	0.367	0.5	nd	4.4	5.5	63.7	3.4	3.3	19.2
60	0.357	0.6	nd	4.6	6.2	60.7	3.8	3.8	20.3
70	0.393	0.5	nd	4.2	5.4	62.4	3.7	3.4	20.3
80	0.364	0.5	nd	4.0	6.0	62.7	3.6	3.4	19.8
90	0.417	0.6	nd	3.9	4.5	66.6	2.7	3.0	18.8

[a] nd, not detected.

DISCUSSION

The firmness of green beans appeared to vary with the conditions of the used preservation process. Sterilized green beans that were preheated at moderate temperatures (50−70 °C) for an appropriate period of time before further processing remained significantly firmer than conventionally processed green beans (Figure 2). This higher retention of firmness of green beans by preheating was already described by many other authors (Chang et al., 1993, 1996; Bourne, 1987; Van Buren et al., 1960). A similar effect is found for a diversity of other vegetables including potatoes and carrots (Sajjaanantakul et al., 1989; Andersson et al., 1994). The only, very evident, change in the cell walls during preheating was a decrease in DM (Table 2). This is consistent with the general idea that the preheating effect can be attributed to demethylation of pectin by endogenous PME during the preheating period (Bartolome and Hoff, 1972; Chang and Chang 1992). The effect on firmness of this reaction becomes evident only after heating at elevated temperatures, like sterilization. The optimal temperature for PME extracted from fresh green bean pods was 55−60 °C (Chang and Chang, 1992). This was also the temperature of the preheating treatment that resulted in the lowest DM after preheating (Table 2). The residual PME activity was low after preheating for 45 min at 60 °C, which indicates that a substantial amount of the PME was denatured at this temperature. The overall activity during the total preheating period, however, may have been very high. Typically, PME binds very strongly to pectin, pure electrostatically, and its activity is tightly controlled during plant development (Jarvis, 1984). Consequently, when a PME has de-esterified a chain segment, it will remain attached there and will be released only by heat or perhaps by PG action. For these reasons, it is indeed very likely that PME is activated during preheating, resulting in removal of methyl esters from the pectin. The most important softening process during steriliza-

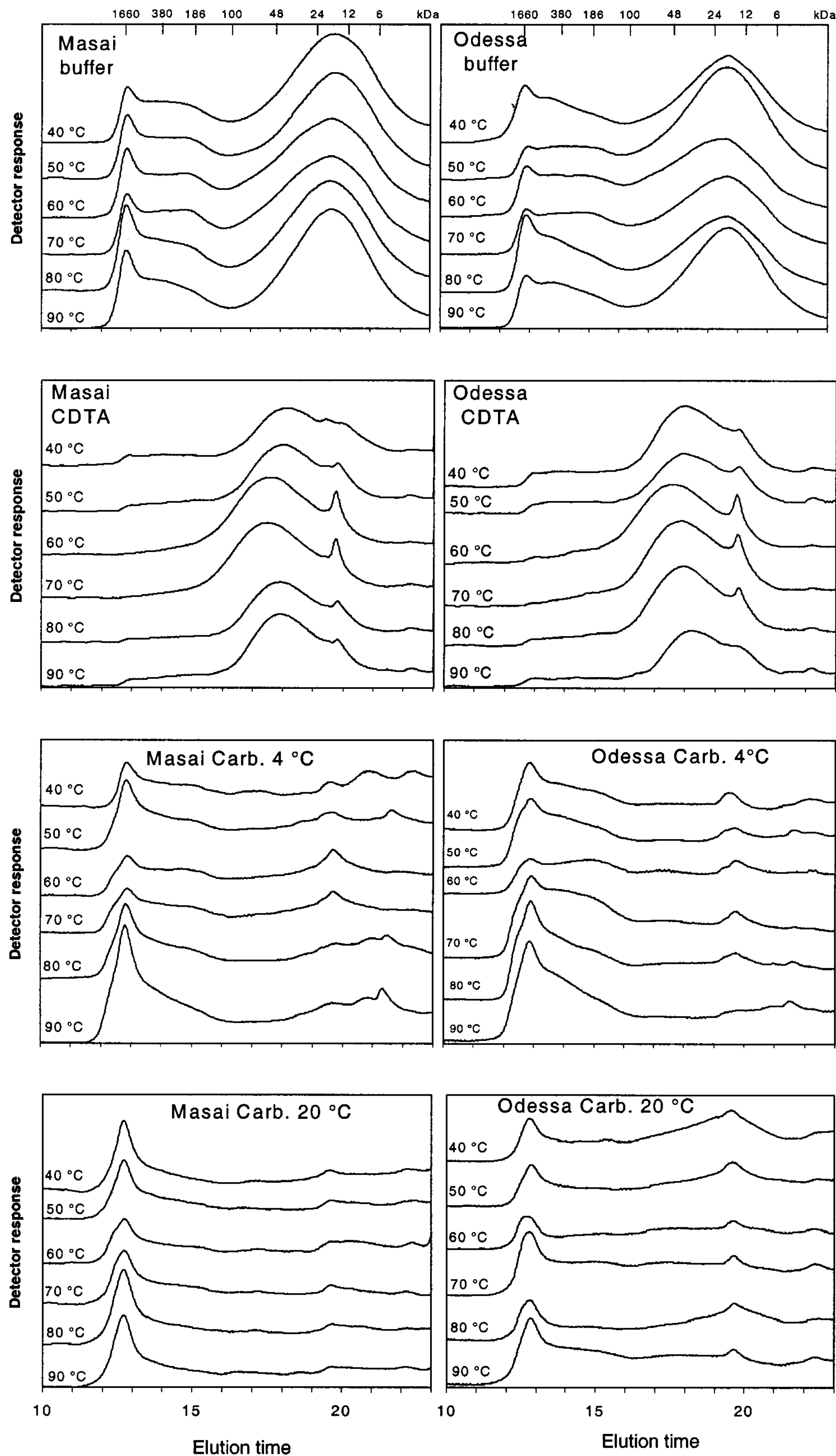


Figure 8. High performance size exclusion chromatography elution patterns of sequential pectic extracts from green bean cv. Masai and Odessa, after processing with different preheating conditions.

tion is believed to be β-elimination (Sajjaanantakul et al., 1989). The reaction rate of β-eliminative degradation depends on temperature, pH, and the presence of a methyl ester at C_6, next to which the chain cleavage occurs. As a consequence, pectin solubilization will occur to a greater extend upon sterilization whenever PME could not act optimally either by too low temperature (low activity) or by too high preheating temperature (denaturation). The increased M_r of the CDTA fractions from the beans preheated at 60 °C indicated that less

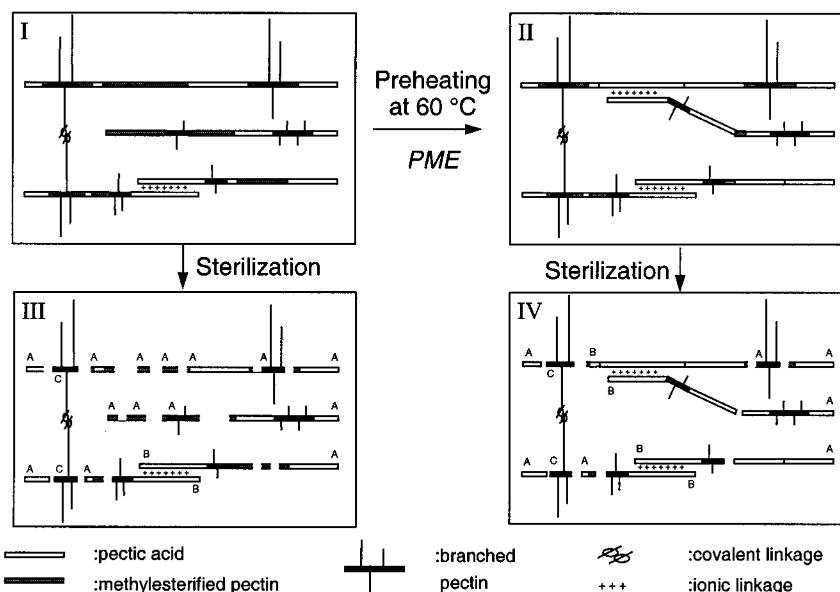


Figure 9. Schematic overview of changes occurring during preheating (II) and sterilization (III and IV) of fresh green beans (I) in relation to the solubilty of pectins. A: buffer soluble; B: CDTA-soluble; C: carbonate-soluble/residue.

cleavage of the pectic chains has occurred in these samples (Figure 8). At the sites were the methyl esters are removed, the pectin (i) can form intermolecular complexes with calcium and (ii) is less heat-labile. This is visualized in a diagram in Figure 9. Both processes result in a higher firmness retention after sterilization. Chang et al. (1993) also tried to model the texture of green beans in relation to the extractability of cell wall polymers during heat treatments. They also showed an increased solubility of uronic acid containing polymers during cooking. However, their extraction procedure was highly destructive, since they used hot water and hot acid. In addition, only a little information was provided about the chemical composition of the cell wall polymers. As a result, their model is highly complex but rather speculative.

The preheating effect was accompanied by an increased yield of buffer and CDTA extracts but had no significant effect on the yield of carbonate extracts. Apparently, the branched pectins, which are recovered predominantly in the carbonate extracts, are flanked by methylated uronic acid residues that are not demethylated by PME during preheating at any of the applied temperatures. This is possibly caused by steric hindrance of PME by the neutral side chains (De Vries et al., 1982).

A small percentage of the sugars in wall polysaccharides contain ferulic acid and related phenolic side groups (Fry, 1988). These structures may be cross-linked by the action of POD and H_2O_2 to form intercellular bridges such as diferulate, thus linking polysaccharides together in a tight network (Ishii, 1997). If there is an enhanced cross-linking of pectins due to POD activity during preheating one could expect that polymers are less easily extracted form the cell wall and that consequently substantially more polymers would be recovered in the carbonate fractions or remain associated with the residue despite the buffer or CDTA fraction. More specifically, at least saponification of ester linkages between the phenolic acid derivative and the sugar alcohol is needed to solubilize the cross-linked polymers. Since we measured no significant increased yields of carbonate or residue fractions, we may deduce that there is very little or no enhanced pectin cross-linking by POD during preheating. Tyrosine units of extensin, an important structural cell wall protein, however, may undergo a similar cross-linking reaction to form isodityrosine cross-links. Extensin is very difficult to extract from the cell wall because isodityrosine cross-links have to be broken. In our study, it is most likely associated with the residue in all samples. Therefore, we cannot exclude that POD has an effect on the texture during preheating by cross-linking of extensins.

After all processing conditions were performed, as applied in the present study, cv. Masai appeared firmer as compared with cv. Odessa (Figure 2). This was accompanied by a somewhat higher percentage of pectin degradation in beans of cv. Odessa. Possibly firmness is simply related with the overall amount of cell wall material, which was higher for cv. Masai (Table 2). A comparable relationship was found for two potato cultivars, which differed with respect to mealiness (Van Marle et al., 1997). Another aspect that might relate to firmness are the properties of the CDTA-soluble pectins. Cultivar Masai seems to contain a higher amount of AUA in the CDTA-soluble pectins. Moreover, the DM and especially the DA are much lower in this fraction of cv. Masai. The DM is well-known to affect the strength of calcium intermediated pectin gels, the normal gel type in cell walls (Jarvis, 1984). Methyl groups prevent calcium binding and make the interjunction segments more flexible. The strength of the calcium gel type depends on the length of the uninterrupted pectate segments that can interact. Acetyl subunits were shown to reduce the binding strength (Kohn and Furda, 1968). With this in mind, it can be hypothesized that the CDTA-soluble pectins, which reflect more or less the calcium-linked pectins, can from stronger, more cohesive, gels in cv. Masai than in cv. Odessa. If CDTA-soluble pectin originates from the middle lamella region, as is often stated, this implies that the cells of cv. Masai may be less easy to separate, resulting in a higher firmness of the tissue.

In conclusion, our results support the general hypothesis that the less softening effect of preheating at moderate temperatures is related to PME. PME is most

likely activated during preheating and demethylates specific regions of the pectic polymers, which results in a decreased breakdown of pectin (Figure 9).

ABBREVIATIONS USED

AIR, alcohol-insoluble residue; AUA, anhydro uronic acid; CDTA, cyclohexane-*trans*-1,2-diamine tetra acetate; CWM, cell wall material; DM, degree of methylation; DA, degree of acetylation; DMSO, dimethyl sulfoxide; DW, dry weight; HPGPC, high performance gel permeation chromatography; M_r , molecular mass relative to pullulan standards; PME, pectin methylesterase; POD, peroxidase; TFA, trifluoroacetic acid.

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