

# Cell Wall Monosaccharide Changes During Softening of Brined Cucumber Mesocarp Tissue

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## ABSTRACT

Blanched, unheated and fermented cucumber mesocarp tissues stored at pH 3.5 had losses in galacturonic acid, galactose, arabinose, and rhamnose from cell walls isolated by sequential extraction with aqueous sodium dodecyl sulfate (SDS) and phenol:acetic acid:water (2:1:1; SDS/PAW). Much smaller amounts of the sugars were solubilized when the cell walls were isolated by extraction with 80% ethanol. The differential solubilization of sugars suggested that a limited degradation of pectic and hemicellulosic substances occurred during cucumber tissue softening. This degradation was readily observed when softened tissue was extracted by SDS/PAW, but not when isolated by the more common ethanol extraction. Similarity in pattern of sugar losses, following low pH storage after three processing treatments, suggested that reactions which resulted in tissue softening and solubilization of cell wall sugars were similar in the three treatments.

Key Words: cucumbers, pickles, monosaccharides, softening, mesocarp

## INTRODUCTION

THE EFFECT of NaCl concentration on the rate of cucumber mesocarp tissue softening has been reported for blanched and nonblanched, acidified tissue and fermented tissue (McFeeters et al., 1989). The effect of multivalent cations, temperature and pH to increase or decrease the rate of softening in blanched mesocarp tissue in the presence of high levels of salt (up to 1.5 M) was subsequently studied to better understand the mechanism of calcium inhibition of tissue softening (McFeeters and Fleming, 1989, 1990, 1991). One conclusion was that softening inhibition by calcium and other metal ions could not be explained on the basis of cross-linking of pectic substances in the cell wall according to the "egg-box" model developed by Grant et al. (1973). A better understanding of the chemical changes responsible for softening under acid conditions is needed. This would help determine the true mechanisms of metal ions inhibiting the rate of softening. An initial step toward understanding the chemistry of the softening process would be to determine whether cell wall carbohydrates become soluble as the tissue softens, and to measure the extent of solubilization of individual monosaccharides if significant solubilization occurs.

Changes in pectic substances related to textural changes caused by processing treatments have been reported for many vegetable tissues, including potatoes (Doesburg, 1961; Hughes et al., 1975; Johnston et al., 1983, Moledina et al., 1981), Japanese radish (Chang et al., 1986; Fuchigami, 1986, 1987a, 1988, 1990), cucumber (Hudson and Buescher, 1985), snap beans (Van Buren et al., 1988), legume seeds (Makino et al., 1987; Uzogara et al., 1990), broccoli, asparagus lettuce, and mustard (Wu and Chang, 1990). Fuchigami (1987b) surveyed softening and pectic changes during cooking of 21 vegetables. Attempts to relate textural changes caused by processing and storage to neutral sugar changes, as well as well as changes in pectic substances, have been limited to cucumbers (Howard

and Buescher, 1990; Tang and McFeeters, 1983), carrots (Plat et al., 1988) and bamboo shoots (Fuchigami, 1990).

For cucumbers, Tang and McFeeters (1983) found no significant changes in sugars hydrolyzed by 2N trifluoroacetic acid from the alcohol-insoluble solids of fermented cucumbers that had undergone limited softening during 6-months storage. Howard and Buescher (1990) also isolated cell walls by ethanol extraction and analyzed neutral sugars by GLC after Saeman hydrolysis. They reported a loss of galactose from cell walls during storage of unpasteurized cucumbers in acid brines similar to that reported by Gross and Wang (1984) during air storage, but the change did not correlate with texture change, which was relatively small. Thus, for cucumbers a clear relationship has not been established between changes in neutral sugars in the cell wall and textural change. Whether this was due to the fact that textural change was not closely related to changes in the solubility of cell wall carbohydrates was uncertain. Possibly, the degree of texture change which occurred was too small to bring about measurable changes in sugars, or the ethanol isolation of the cell wall was not a suitable isolation technique to extract polysaccharides that may have been degraded only to a limited degree.

The objective of this work was to determine whether softening of cucumber mesocarp tissue which had softened to a greater extent than that of previous studies of Tang and McFeeters (1983) and Howard and Buescher (1990) would be accompanied by measurable changes in solubility of cell wall carbohydrates. Since previous efforts using 80% ethanol isolation of cell walls did not show changes in sugars related to textural changes, two methods for cell wall preparation were compared: 80% ethanol extraction and a serial sodium dodecyl sulfate/phenol:acetic:water (2:2:1) extraction procedure described by Jarvis (1982). If limited degradation of some polysaccharides occurred, they might be soluble in the aqueous SDS used in the Jarvis (1982) procedure, but not soluble in 80% ethanol. Three processing treatments were investigated: blanched to inactivate endogenous enzymes; unheated so that endogenous enzymes would remain active; and fermented with *Lactobacillus plantarum* prior to softening.

## MATERIALS & METHODS

CUCUMBERS (cv. Calypso) were grown locally using standard cultivation procedures. Size 3B fruit (44-51 mm diameter) were harvested, washed, and sliced to 8 mm thickness perpendicular to the long axis. The slices were peeled and the mesocarp tissue from the three carpels cut off and used for the experiment. Tissue ( $\approx 15$ mm) from the stem and blossom ends of each fruit was discarded.

### Treatment 1, blanched

Mesocarp pieces ( $\approx 400$  g/batch) were placed in four layers in a stainless steel wire rack. Each layer was separated by a divider. The pieces were blanched for 3 min in boiling distilled water and then cooled for 2 min in room temperature distilled water. Batches ( $120 \pm 0.1$ g) of mesocarp were placed into six 240 mL jars and covered with a brine consisting of 0.20M acetic acid, 400 ppm SO<sub>2</sub> as Na metabisulfite, and 2.0M NaCl (density 1.0755 g/mL). The blanched mesocarp tissue was covered with 129.4g of brine (120 mL) to give an equal volume of tissue and cover brine. The jars were sealed and

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# CELL WALL CHANGES IN CUCUMBER TISSUE. . .

Table 1—Firmness of fresh, blanched, and fermented cucumber mesocarp tissue. Brined samples were incubated at 45°C\*

	Incubation time, days	Firmness, N	Standard error	CV, %
Fresh tissue		8.7	0.9	10.3
Blanched tissue		10.1	1.9	19.0
Blanched treatment	0	9.8	1.3	13.1
	7	0.4	0.1	32.4
	35	0.3	0.1	35.2
Unheated treatment	0	10.4	1.6	15.4
	7	4.6	1.6	35.3
	35	0.8	0.3	38.4
Fermented treatment	0	8.2	1.7	21.2
	7	1.4	0.4	28.4
	28	0.8	0.3	33.0

\* The mean firmness and standard errors of the firmness measurements were calculated over duplicate jars for each sampling time.

initially placed overnight in a 17°C incubator to allow the salts to equilibrate. After equilibration, two jars were opened immediately. The firmness of tissue samples was measured. Samples were then blended and cell walls isolated from tissue from each jar by each of the two extraction procedures. The remaining four jars were placed in a 45°C incubator to allow rapid softening. Duplicate jars were sampled after 7 and 35 days' incubation at 45°C.

## Treatment 2, unheated

Jars of mesocarp tissue were prepared and sampled exactly as in treatment 1 except that the tissue was not blanched.

## Treatment 3, fermented

Two jars (946 mL capacity) were each filled with 480 g of unheated mesocarp and 480 mL of brine. The brine contained 0.107 M acetic acid, 0.072 M NaOH and 2.0 M NaCl (density 1.0755). Each jar was inoculated with 1.25 mL of a 24 hr culture of *Lactobacillus plantarum* WSO grown on MRS broth (DeMan et al., 1960), closed and placed in a 30°C incubator to ferment. At the end of fermentation (7 days), the contents of each of the two jars were used to fill three 240 mL jars with equal weights of tissue pieces and fermentation brine. Two 240 mL jars, one from each of the 946 mL fermentation jars, were immediately opened, firmness measurements were made on the tissue pieces, and the cell walls isolated from blended samples of the tissue pieces. The remaining four jars were incubated at 45°C and the mesocarp tissue allowed to soften. Duplicate jars were analyzed 7 and 28 days after placement in the 45°C incubator.

## Tissue firmness measurement

The force required to puncture tissue pieces was recorded for 15 tissue pieces from each jar using a 0.315 cm diameter punch on an Instron Universal Testing Machine (Thompson et al., 1982).

## Cell wall isolation by 80% ethanol (McFeeters and Armstrong, 1984)

Liquid was drained from the tissue pieces. The pieces were blended with a Tekmar homogenizer. A sample of the stirred slurry (30g) was blended with 5 volumes of 95% ethanol and filtered on a Büchner funnel through a Whatman No. 1 filter paper. The insoluble residue was resuspended in 80% ethanol, blended and refiltered. The cell wall

residue was resuspended in 2.5-fold (v/w) acetone relative to the starting slurry, blended again, and filtered (as dry as possible) on the Büchner funnel. The cell walls were dried in a vacuum oven at 25°C, weighed, and stored in a desiccator until analysis.

## Aqueous SDS/PAW cell wall isolation (Jarvis, 1982)

To 80 g of stirred tissue slurry in a 250 mL centrifuge bottle, 26.7 mL 2.0% aqueous sodium dodecyl sulfate was added. After mixing, the suspension was centrifuged for 10 min at 8,000 rpm. Any floating cell wall particles were recovered by decanting the supernatant through a Miracloth filter. Water and 2.0% SDS were added back to the cell wall residue to give a total weight of 106.7g and 0.25% final SDS concentration. The SDS solution was mixed well with the cell wall, centrifuged, and the supernatant decanted again. Water was added back to the centrifuge tube to bring the weight back to 106.7g. The mixing and centrifugation steps were repeated. Water washing was done a total of three times. After the third water wash, the insoluble residue was filtered on a Büchner funnel on Miracloth to reduce the volume of the cell wall residue. Phenol:acetic acid 2:1 (w/w) was added to the residue to give a final concentration of 2:1:1 phenol:acetic acid:water and the mixture centrifuged and decanted. The residue was washed three times with water to remove most of the phenol and acetic acid. The insoluble material was suspended in 50 mL acetone and filtered. It was resuspended in 75 mL acetone and filtered again. The PAW extracted cell wall was placed in a vacuum oven, dried, and weighed. The cell wall samples were stored in a desiccator until analysis.

## Cell wall hydrolysis and sugar analysis

The procedure used for analysis of cucumber cell wall monosaccharides was that of McFeeters and Lovdal (1987). The hydrolysis and neutral sugar analysis were based upon the procedure of Blakeney et al. (1983) for GLC analysis of the alditol acetate derivatives. Uronic acid determinations were done using the 3,5-dimethylphenol reagent (Scott, 1979). Triplicate cell wall hydrolyses and analysis of the sugars were done on duplicate jars of tissue at each of the three sampling times.

## RESULTS

NO EVIDENCE of microbial spoilage was observed in any samples, as indicated by the absence of any gas formation, turbidity, or pH change during storage. This was expected since the addition of 200 ppm SO<sub>2</sub> in nearly identical conditions of acid and salt prevented the growth of microorganisms in previous experiments (McFeeters et al., 1989). Microbial stability of the fermented samples was based upon the fermentation of sugars to lactic acid and the high salt concentration (Fleming et al., 1983).

The blanched samples had lost 96% of the initial firmness after 7 days' incubation at 45°C (Table 1). For fermented mesocarp tissue, the loss was 83%. A small amount of additional texture loss occurred at the final sampling time in each case. The nonblanched tissue softened more slowly, losing 56% of the initial firmness in 7 days and 92% after 28 days.

The zero time cell wall sugar composition and estimates of analytical errors were similar for the 80% ethanol extraction method (Table 2) and for the SDS/PAW extraction procedure

Table 2—Monosaccharide composition of hydrolyzed cell walls from the zero time samples of three treatments of brined cucumber mesocarp tissue\*

	Blanched			Unheated			Fermented		
	Mean	Standard error	CV, %	Mean	Standard error	CV, %	Mean	Standard error	CV, %
Rhamnose	0.36	0.01	2.0	0.32	0.04	11.9	0.31	0.02	6.1
Fucose	0.12	0.06	52.6	0.11	0.06	50.1	0.12	0.02	15.8
Arabinose	1.08	0.03	2.4	1.06	0.05	4.9	0.95	0.03	3.6
Xylose	2.62	0.08	2.9	2.17	0.07	3.1	2.00	0.07	3.4
Mannose	1.46	0.06	4.2	1.39	0.04	2.6	1.32	0.02	1.5
Galactose	6.87	0.11	1.6	6.09	0.38	6.1	5.36	0.13	2.4
Glucose	14.94	0.86	5.7	13.37	0.97	7.2	12.36	0.29	2.3
Uronic acid	6.02	0.24	3.9	6.30	0.44	7.0	5.28	0.13	2.4
Total sugars	33.48			30.81			27.71		

\* Cell walls were isolated by 80% ethanol extraction. Concentrations are  $\mu$ mole monosaccharide/g fresh weight of cucumber mesocarp tissue.

Table 3—Monosaccharide composition of hydrolyzed cell walls from the zero time samples of three treatments of brined cucumber mesocarp tissue\*

	Blanched			Unheated			Fermented		
	Mean	Standard error	CV, %	Mean	Standard error	CV, %	Mean	Standard error	CV, %
Rhamnose	0.30	0.02	7.6	0.37	0.04	9.4	0.34	0.01	3.5
Fucose	0.14	0.00	3.4	0.16	0.02	11.6	0.13	0.01	6.8
Arabinose	0.93	0.03	3.6	0.98	0.06	6.3	0.85	0.01	1.6
Xylose	2.37	0.20	8.2	2.41	0.10	4.2	2.28	0.03	1.5
Mannose	1.36	0.05	3.6	1.47	0.07	5.0	1.38	0.09	6.3
Galactose	6.63	0.17	2.5	6.85	0.41	6.0	5.92	0.08	1.3
Glucose	14.51	0.55	3.8	15.24	0.80	5.2	13.99	0.44	3.1
Uronic acid	6.41	0.44	6.9	6.35	0.58	9.1	5.62	0.14	2.5
Total sugars	32.65			33.83			30.51		

\* Cell walls were isolated by SDS/PAW extraction. Concentrations are  $\mu$ mole monosaccharide/g fresh weight of cucumber mesocarp tissue.

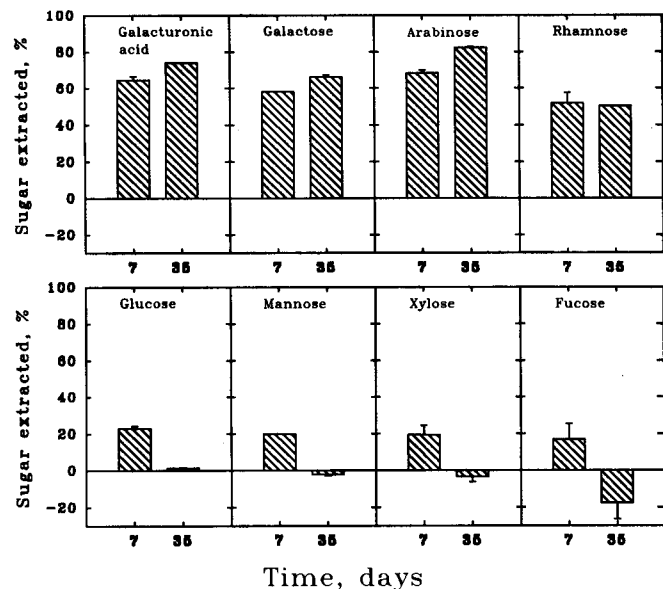


Fig. 1—Changes of monosaccharides after hydrolysis of cell walls isolated by SDS/PAW extraction of blanched cucumber mesocarp tissue after 7 and 35 days' incubation at 45°C in 1.0M NaCl at pH 3.5. Percentages of sugars extracted are expressed relative to the monosaccharide composition of the cell walls at zero time (Table 3). Each bar represents analysis of duplicates. Standard error indicated by error bars.

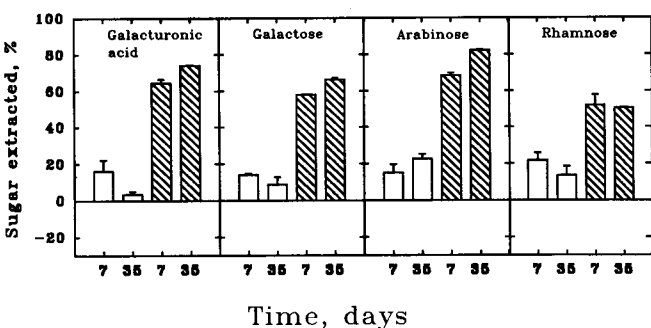


Fig. 2—Comparison of losses of galacturonic acid, galactose, arabinose, and rhamnose from cell walls isolated by 80% ethanol and SDS/PAW extraction of blanched cucumber mesocarp tissues after 7 and 35 days' incubation in 1.0M NaCl at pH 3.5. Percentages of sugars extracted are expressed relative to the monosaccharide composition of the cell walls at zero time (Tables 2 and 3). □ 80% ethanol extraction; ▨ SDS/PAW extraction. Each bar is the mean of duplicates. Standard error indicated by error bars.

(Table 3). Initially, when the tissues were firm, the sugar composition was similar whether isolated by ethanol extraction or by SDS/PAW. Ethanol will precipitate polysaccharides other

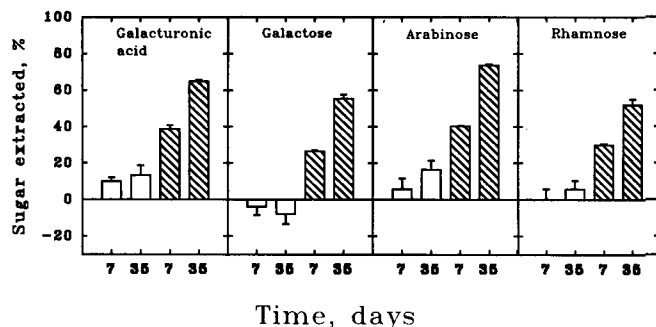


Fig. 3—Comparison of losses of galacturonic acid, galactose, arabinose, and rhamnose from cell walls isolated by 80% ethanol and SDS/PAW extraction of unheated cucumber mesocarp tissues after 7 and 35 days' incubation in 1.0M NaCl at pH 3.5. For legends see Fig. 2.

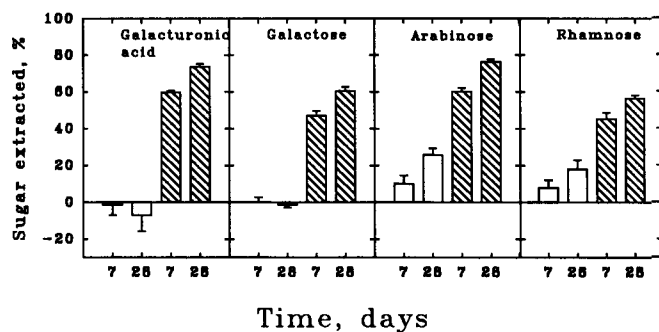


Fig. 4—Comparison of losses of galacturonic acid, galactose, arabinose, and rhamnose from cell walls isolated by 80% ethanol and SDS/PAW extraction of fermented cucumber mesocarp tissues after 7 and 28 days' incubation in 1.0M NaCl at pH 3.5. For legends see Fig. 2.

than short oligomers, usually with DP < 5. That the cell wall composition from ethanol extraction was not changed when the aqueous SDS/PAW extraction method was used, indicated that in firm tissue the cell wall polysaccharides were insoluble in an aqueous extraction medium as well as in ethanol.

The amounts of solubilized sugars relative to the initial levels for the eight cell wall monosaccharides after 7 and 35 days of softening at 45°C for the blanched mesocarp tissue samples were compared (Fig. 1). Monosaccharides associated with the pectic substances, namely galacturonic acid, galactose, arabinose, and rhamnose (Fig. 1), showed reductions of about 50–80% in the insoluble cell walls isolated from softened tissues. In contrast, glucose, mannose, xylose, and fucose, monosaccharides associated primarily with hemicellulose and cellulose did not show consistent losses as tissue softened. These sugars were 15–20% solubilized after 7 days, but this was not observed after 35 days. No consistent pattern of changes for these

sugars was observed for the unheated and fermented treatments.

Comparisons of losses of galacturonic acid, galactose, arabinose, and rhamnose from cell wall isolated by the ethanol vs SDS/PAW extraction methods are shown in Fig. 2 to 4 for the blanched, unheated and fermented treatments, respectively. The losses of sugars observed for the ethanol-isolated cell walls as tissue softening proceeded were small and inconsistent for all three treatments. There may have been some extraction of these sugars as the blanched tissue softened (Fig. 2), but the changes were not consistently greater after 35 days as compared to 7 days. On the other hand, SDS/PAW isolation of the cell walls resulted in solubilization of a large proportion of all four sugars as tissue softening proceeded. With the exception of rhamnose in the blanched samples, more of the sugars were lost from the longer incubation period samples. The unheated samples were of particular interest in this respect. The differences between the 7 and 35 day sugar losses were greater than for the other treatments. This relates to the fact that after 7 days the loss in tissue firmness was smaller for the unheated tissue than the other two treatments (Table 1). The other treatments were already very soft (<2 N) after 7 days so there was <1 N additional loss of firmness by 28 or 35 days and a correspondingly smaller increase in sugars extracted at the later sampling times. Thus, the extractability of sugars from the cell wall by the SDS/PAW technique appears to parallel the loss in tissue firmness.

## DISCUSSION

TISSUE was allowed to soften in the presence of a high salt concentration, as was done previously (McFeeters et al., 1989). Despite undergoing extensive softening, cell walls did not show consistent changes in monosaccharide composition when the ethanol isolation procedure was used. Thus, this extraction technique does not demonstrate the occurrence of cell wall degradation, though some degradation likely occurred. Lack of sugar changes was also reported earlier by Tang and McFeeters (1983) when sugars hydrolyzed by 2N trifluoroacetic acid were analyzed in ethanol isolated cell walls from stored, fermented cucumbers. In contrast, the SDS/PAW isolation procedure showed large losses of galacturonic acid, galactose, arabinose, and rhamnose from the cell walls of softened tissue compared to cell walls from firm tissue. The changes in monosaccharide composition which are evident in the cell walls isolated by the SDS/PAW method indicated that some limited degradation of cell wall components occurred as tissue softened. If the cell wall polysaccharides had been degraded to monomers or small oligomers, loss of sugars would also have been evident in the ethanol isolated walls because degradation products of that size range would be soluble in 80% ethanol.

These results differed from previous analyses of heat-softened tissue because they demonstrated that sugars in the cell wall other than galacturonic acid become extractable in large amounts. Rhamnose has been shown to occur in the main chain of pectin polymers, while arabinose and galactose occur in pectic substances as short side chains (Fry, 1988). Therefore, the pattern of sugar losses from soft mesocarp tissue indicated that some breakdown or rearrangement of pectic substances occurred during softening that resulted in increased solubility. Possibly, some of the neutral sugars that became extractable were from galactans or arabinogalactans that were not directly attached to pectin polymers (Fry, 1988). Cellulose and polysaccharides containing primarily xylose and mannose were not degraded to the point that solubilization occurred. That the pattern of sugar losses was the same for all three treatments, suggested that similar degradation reactions probably occurred whether the cucumber tissue was blanched, and/or fermented, or not. This work did not directly address which reaction(s) may have caused tissue softening. Observations were reported of the solubilization of pectic substances during tissue softening

(Doesburg, 1961) and that polygalacturonic acid hydrolysis would occur faster than most neutral polysaccharides at pH 3.5 (Smidsrod et al., 1966) where these experiments were carried out. Based on those, possibly limited acid hydrolysis of the pectic substances caused tissue softening in our experiments. However, an argument against acid hydrolysis as the major softening reaction is the observation that for softening under similar salt and pH conditions the entropy and enthalpy of activation are not those expected if rate-limiting degradation reactions were acid hydrolysis of glycosidic bonds (McFeeters and Fleming, 1990).

Thus, softening results in degradation of cell wall structure in a way that causes solubilization of a large fraction of galactose, arabinose and rhamnose, in addition to uronic acid in the wall by SDS/PAW extraction. These results indicate a need for further chemical characterization of the tissue softening process. The next step should be to develop procedures to isolate, fractionate and characterize the oligosaccharides or polysaccharides which become soluble in SDS/PAW as a consequence of softening. An important issue would be to determine whether the rate-limiting softening reaction involves the hydrolysis of glycosidic linkages or if it involves disruption of noncovalent interactions of the cell wall components.

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