Changes in Pectin and Cellulose during Processing

R. F. McFeeters[1]

INTRODUCTION

Polysaccharides provide much of the structure which gives a wide variety of foods desirable textural properties. Cellulose, pectin, and hemicellulose are the major polysaccharide components in the cell wall of all plant foods. Due to the complexities of these polysaccharides, the even greater complexity of the plant cell wall, and numerous experimental difficulties in working with these polysaccharides, much remains to be learned in establishing detailed structure–function relationships both for biological and technological functions. However, there has been rapid progress in understanding the physical and chemical properties of polysaccharides in recent years. As new information and new techniques are applied more intensively to problems of interest to food technologists, we are likely to see considerable progress in our understanding of the chemistry of food texture. The purpose of this chapter is to review recent developments in the chemistry of plant cell wall polysaccharides, particularly pectin and cellulose, as they may relate to the texture of fruits and vegetables.

CELL WALL STRUCTURE

There have been major advances in our understanding of plant cell wall structure during the past 15 years. This progress has been made

[1]Food Fermentation Laboratory, Agricultural Research Service, U.S. Department of Agriculture and North Carolina Agricultural Research Service, Department of Food Science, North Carolina State University, Raleigh, NC 27695.

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possible by improvements in the methods available to analyze complex polysaccharides. For example, Waeghe *et al.* (1983) have recently published procedures which require only 5 μg of starting material to analyze sugar composition and linkage patterns in cell wall polysaccharides by using capillary gas chromatography and mass spectroscopy. Advances are also being made in mass spectrometry of underivatized carbohydrates (Dell *et al.* 1983) and structural analysis of complex carbohydrates using both ^{13}C nuclear magnetic resonance (NMR) (Barker *et al.* 1982) and proton NMR (Dabrowski *et al.* 1982), so that it is now possible to do sequence analysis of complex polysaccharides (McNeil *et al.* 1982). Darvill *et al.* (1980) and McNeil *et al.* (1984) have presented detailed reviews of studies of cell wall structure, particularly of the work in Albersheim's laboratory. Table 16.1 shows the major components in suspension-cultured sycamore cell walls.

TABLE 16.1. POLYMER COMPOSITION OF THE WALLS OF SUSPENSION-CULTURED SYCAMORE CELLS

Wall component	Weight (%) of cell wall
A. Pectic polysaccharides	34
Rhamnogalacturonan I	7
Homogalacturonan	6
Arabinan	9
Galactan and possible arabinogalactan	9
Rhamnogalacturonan II	3
B. Hemicelluloses	24
Xyloglucan	19
Glucuronoarabinoxylan	5
C. Cellulose	23
D. Hydroxyproline-rich glycoprotein	19

From Darvill et al. (1980).

Cellulose has a partially crystalline, fibrous structure to provide the basic structural strength of the cell wall. Pectic polysaccharides and hemicelluloses constitute an amorphous matrix around the cellulose fibers. Specific structural roles for the individual fractions of these matrix components remain to be defined. Lamport (1965) originally suggested that hydroxproline-rich glycoproteins control the extension of cell walls during plant growth and suggested the name "extensin" for these glycoproteins. However, their functional role also remains to be defined (Lamport 1980).

All plant cell walls contain the four major components listed in Table 16.1. Monocot plants differ from dicots principally because they have a much lower content of pectic substances and a higher content of xylans. From the viewpoint of trying to define basic principles of cell wall structure and function, Darvill *et al.* (1980) have emphasized the similarities of cell wall polysaccharides among higher plants.

However, if we are to explain the wide variety of textures in fruit, vegetable, and cereal products, I suspect food technologists will also have to appreciate the considerable dissimilarities in cell wall composition which appear to occur. Table 16.2 is a compilation of the sugar and uronic acid composition of a variety of fruit and vegetable cell walls which have recently been analyzed by Voragen *et al.* (1983) and Selvendran and co-workers (Selvendran 1983). Within this group of products, there are large variations in the relative amounts of each sugar present in the cell wall, with a range of 2.8-fold for glucose to 17.7-fold for xylose. This is an indication of the magnitude of variability in cell wall components that occur in foods. There may also be substantial variability in the size, branching, and sugar composition within the polysaccharide structure. The pectic substances will vary in degree of carboxyl methylation. In addition, there will be differences in the amount and types of proteins and lignin present.

It is not surprising, in view of the many possibilities for variation in cell wall structure, that there is much to be learned about structure–function relationships. A recent development which may add another useful tool to the techniques for understanding polysaccharide changes has been the use of 4-methylmorpholine *N*-oxide (MMNO) to dissolve cellulose and cell walls. Joseleau *et al.* (1981) showed that cellulose dissolved in MMNO underwent little or no degradation, as indicated by methylation analysis of the dissolved polymer. They also found that primary cell walls from suspension-cultured plant cells could be almost completely dissolved in MMNO and then remain in solution when diluted with dimethyl sulfoxide. This group has recently utilized MMNO to partially fractionate cell wall components (Chambat *et al.* 1984). The ability to dissolve these polymers without degradation offers the opportunity to fractionate complex mixtures of polysaccharides in ways that were previously impossible.

By far, the greatest amount of research on the role of cell wall components in food texture has been done on pectic substances. As a result, pectin chemistry will be the major focus of this review. Cellulose has received little attention. Hemicellulose polysaccharides and cell wall structural proteins have not been available in sufficient quantity to even seriously begin investigations into their role in texture and texture changes in fruits and vegetables.

SUGAR COMPOSITION, SIZE, AND SHAPE OF PECTIN MOLECULES

Pectin molecules have a backbone of linear chains of galacturonic acid residues in α-1,4-glycosidic linkages. Rees and Wight (1971) de-

TABLE 16.2. SUGAR COMPOSITION OF CELL WALLS FROM VARIOUS FRUITS AND VEGETABLES[a]

	Reference[b]	6-Deoxyhexose[c]	Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic acid	Total[d]
Potatoes	1	20.4	46.1	16.5	8.3	284	315	270	960
Runner beans	1	19.3	35.1	17.3	15.7	74.8	314	367	843
Apples	1	19.6	122.8	33.3	43.0	56.8	227	328	830
Apples	2	18	103	58	20	67	251	239	756
Raspberries	2	6	23	45	15	22	158	148	417
Strawberries	2	9	47	35	19	44	147	274	575
Cherries	2	8	91	12	11	36	124	308	590
Papaya	2	8	12	22	26	48	268	357	741
Mango	2	9	41	55	13	36	236	322	712
Pear	2	10	42	129	16	31	206	171	605
Carrots	2	11	67	9	16	78	259	312	752
Cucumber	2	7	22	36	20	62	291	216	654
Onions	2	9	26	13	—	201[e]	210	229	688
Pineapple	2	2	101	159	20	65	351	66	764
Ratio (high/low)		10.2	10.2	17.7	5.2	12.9	2.8	5.6	

[a] Neutral sugars analyzed after Saeman hydrolysis. Uronic acid measured colorimetrically. Results expressed as μg/mg of isolated cell wall.
[b] 1, Selvendran (1983); 2, Voragen *et al.* (1983).
[c] Combination of rhamnose and fucose.
[d] The totals do not include protein and lignin which often are present in substantial quantities in cell wall preparations. See Voragen *et al.* (1983).
[e]. Combination of mannose and galactose.

fined the major conformational characteristics of polygalacturonate chains. The uronic acid residues have a helical arrangement with exactly three monomers per turn of the helix. The glycosidic oxygen atom is in an axial position relative to the plane of the monomer. This has the effect of forming a buckled ribbon conformation (Rees 1977) which is quite rigid because any rotation about the glycosidic bonds will cause contact between groups on adjacent residues. The somewhat buckled shape of the molecule is indicated by the fact that the residue length projected on the helix axis is only 4.35Å compared to a residue length of 5.15 Å for glucose residues in cellulose, which has a more extended ribbon conformation (Gardner and Blackwell 1974).

A general feature of all gel-forming polysaccharides is that they have structural irregularities which reduce the regularity of interchain associations (Powell *et al.* 1982). If this did not occur, polysaccharides such as pectin, alginate, or carageenan would probably form condensed, insoluble precipitates rather than hydrated gels with variable firmness and rigidity. Irregularities in the structure of pectin are caused by variable methylation of the carboxyl groups of galacturonic acid residues, neutral sugar side chains, and occasional rhamnose residues in the main chain of the molecule. Using conformational calculations, Rees and Wight (1971) found that rhamnose causes a pronounced kink in a chain of galacturonic acid residues (Fig. 16.1). It is still not known whether rhamnose in pectin has an α- or β-configuration, but both configurations result in formation of a kink. The distance between rhamnose kinks is not known with certainty. Indeed, it could be an important factor in pectin variability among species. However, Powell *et al.* (1982) prepared block polymers from citrus, apple, and sunflower pectin by hydrolysis in 0.25 *M* H_2SO_4 at 100°C for 3 hr. Based upon the relative instability of rhamnosyl glycosidic linkages compared to galacturonic acid bonds, they suggested that hydrolysis occurred primarily at rhamnose residues. Since the hydrolyzed oligomers had a narrow size range and were about 25 residues long, it suggests that, in these pectins at least, rhamnose residues are located about 25 galacturonic acid residues apart.

Except for highly degraded samples, neutral sugars have always been found in pectin. It is likely that commercial pectin preparations, which have been used in almost all physicochemical studies on pectin, will generally contain less neutral sugars than native pectin because hot acid is normally used for commercial extraction. Table 16.3 shows the data of Voragen *et al.* (1983) for the neutral sugars in pectin extracted from the alcohol-insoluble solids of fruits and vegetables by 5 m*M* EDTA in cold 50 m*M* NaOH. Included for comparison are the data of Fishman *et al.* (1984A) for a dialyzed sample of commercially

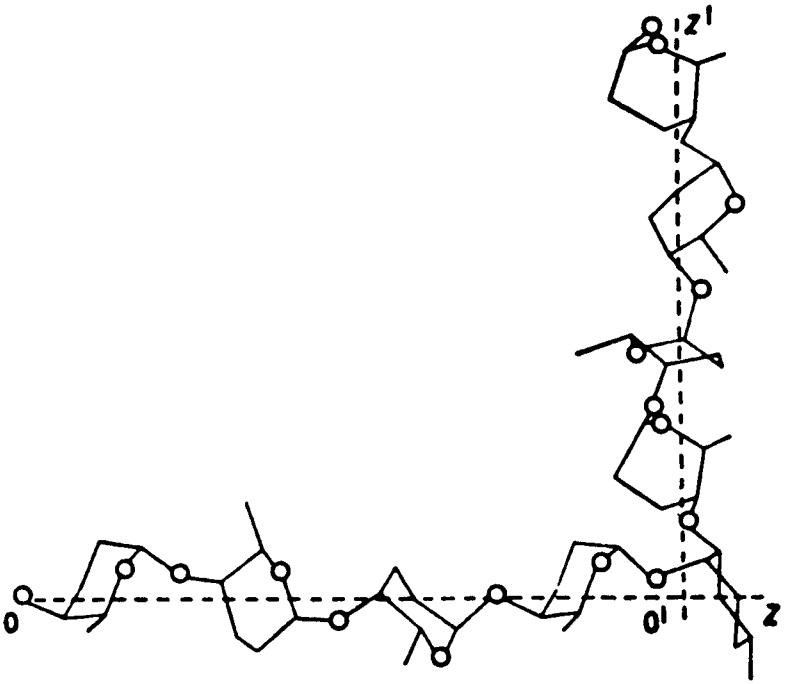

FIG. 16.1. Effect of inserting a single β-L-rhamnose unit, linked through its 2 position, into a galacturonan chain. The rhamnose residue is located at the point where the chain kinks.
From Rees and Wight (1971).

prepared pectin. The most notable difference between the commercial sample and the other pectin extracts is the higher proportion of rhamnose, suggesting a larger proportion of kinks in the uronic acid chains. Just as was the case for total cell wall sugars, the relative proportions of sugars from the different tissues varied over a severalfold range. Studies on the effects of neutral sugar composition upon the physical properties of pectin remain to be done.

The molecular weight of pectin preparations has been reported to vary from 6100 for a commercial sample of citrus polypectate (Fishman *et al.* 1984A) to 366,000 for pectin isolated from unripe peaches (Shewfelt *et al.* 1971). Estimates of pectin molecular weights have been limited by two serious problems. The first is that pectin is normally tightly held in the cell wall matrix. Therefore, it is likely that any extracted pectin which can be used for molecular weight determinations is partially degraded. However, there is no way at present to assess the extent of any degradation which occurs during extrac-

TABLE 16.3. SUGAR COMPOSITION (MOL %) OF A COMMERCIAL PECTIN SAMPLE AND THE PECTIN FRACTIONS ISOLATED FROM CELL WALLS OF FRUITS AND VEGETABLES

Commodity	6-Deoxyhexose[a]	Arabinose	Xylose	Mannose	Galactose	Glucose	Uronic acid
Commercial pectin[b]	7.00	2.03	1.12	1.32	8.49	2.03	78.01
Raspberries[c]	1.6	3.4	2.6	5.5	3.5	4.9	78.5
Strawberries	2.3	9.5	1.5	—	8.4	6.9	71.4
Cherries	2.3	20.6	1.4	2.1	5.9	1.5	66.3
Papaya	3	4.5	4.5	1	6.7	1	79.3
Pineapple	0.7	29.8	36.5	0.6	9.7	4.2	18.4
Mango	1	15.5	1.8	0.6	4.4	2.7	74
Apple	1.4	23.3	2	0.4	4.1	1.3	67.5
Pear	1.8	21.5	2.5	0.8	3.2	11	59.2
Carrots	1	7.9	—	—	11.8	2	77.4
Cucumber	0.6	5.8	2.2	—	11	1.8	78.7
Onions	0.6	3.8	2.3	0.6	40	4.4	48.3

[a] Combined rhamnose and fucose.
[b] From Fishman *et al.* (1984A). The 6-deoxyhexose is only rhamnose.
[c] All fruit and vegetable data from Voragen *et al.* (1983).

tion. The second problem is that pectin aggregates in aqueous solution (Sorochan *et al.* 1971; Fishman *et al.* 1984A). The extent of aggregation can be affected by the extent of methylation, presence of neutral sugars, pH, and ionic strength of the medium. Aggregation will result in overestimating molecular weights using viscometry, ultracentrifugation, light scattering, or osmometry.

Fishman *et al.* (1984A) determined the number average molecular weight for several pectin samples by using ^{13}C NMR spectroscopy to measure the galacturonic acid content and a reducing end group assay to determine the number of pectin molecules. Neither of these determinations should be affected by the aggregation of pectin. Molecular weights ranging from 6100 to 15,200 were obtained. The accuracy of this procedure would be expected to decrease as molecular weights increase because the proportion of reducing groups in a sample will become very small. Fishman *et al.* (1984B) found a molecular weight of 6900 using osmometry in 0.05 *M* NaCl for the same polypectate sample for which a 6100 molecular weight had been obtained by chemical determination. This indicated that 0.05 *M* NaCl prevented aggregation of this sample. Unfortunately, there is not a single solvent system which will assure the disaggregation of pectin samples for physical determinations of molecular size.

PECTIN METHYLATION

Table 16.4 indicates that the extent of methyl esterification of pectin can vary over a wide range in fruit and vegetable tissues. It also indicates that two procedures which are used to measure esterification can give quite different results. However, regardless of the analytical procedure employed, in no case has completely esterified or deesterified pectin been isolated from cell walls. Few details of the mechanism of methylation in vivo are known. At one time, it was believed that galacturonic acid methyl ester was incorporated into the galacturonan chain during synthesis (Albersheim and Bonner 1959), but more recent evidence appears to favor methylation after polymer formation (Kauss and Hassid 1967). *S*-Adenosylmethionine is the source of the methyl groups in pectin (Kauss *et al.* 1969). It is not known whether, as a general rule, galacturonic acid residues are methylated randomly or by some defined pattern. However, recent analysis of the statistical distribution of methyl groups in apple pectin indicates a random distribution of esterified carboxyl groups (DeVries *et al.* 1983B). Despite the high level of pectinesterase present in the albedo of citrus fruits, a random distribution of methyl

groups was also found in pectin from lemon (DeVries *et al.* 1984). Since pectinesterases from higher plants do a sequential hydrolysis of methyl groups from pectin to give blocks of demethylated residues (Rexova-Benkova and Markovic 1976), these results suggest that galacturonic acid residues may be methylated by a random mechanism.

Chemical procedures have been developed for both esterification and deesterification of isolated pectin. The most common methylation techniques are esterification in acidified methanol (Heri *et al.* 1961) or use of diazomethane (Pfeffer *et al.* 1981). There is recent evidence that diazomethane can also form methyl esters on the secondary hy-

TABLE 16.4. DEGREE OF ESTERIFICATION OF URONIDES ESTIMATED FROM METHANOL RELEASE AND COPPER BINDING BY THE PECTIN FRACTION

	Methanol release	Copper binding
Raspberries	20[a]	55
Strawberries	60	88
Cherries	36	44
Papaya	56	68
Pineapple	22	25
Mango	78	79
Apple	72	81
Pear	51	61
Carrots	45	63
Cucumber	51	73
Onions	50	78

From Voragen et al. (1983).
[a] Percentage esterification.

droxyl groups of galacturonic acid residues in pectin (Fishman *et al.* 1984A). Deesterification is done either by base hydrolysis at 0°C (Powell *et al.* 1982) or acid hydrolysis at room temperature (Speiser *et al.* 1945). Base hydrolysis is much more rapid, but chain scission may occur unless the temperature is carefully controlled. All of the chemical techniques result in a random distribution of methyl groups. The only selectivity that has been observed is that when a partial alkaline deesterification of pectin is carried out by reducing the temperature and limiting the reaction time, the free carboxyl groups released will be located between methyl groups whenever possible. The consequence of this pattern is that at 50% esterification of the carboxyl groups, there will be alternating free and methylated carboxyl groups (Deuel *et al.* 1953; Katchalsky and Feitelson 1954).

PECTIN IONIZATION AND ION BINDING

Many of the properties of pectins in plant cell walls and in processed foods are greatly affected by the ionization of free carboxyl groups and by the binding of metal ions. The experimental pK_a values of the free carboxyl groups of pectic acid (≈0.1% solution) vary from 3.6 to 4.1 as the pectic acid is titrated from the fully protonated form to the completely deprotonated form (Fig. 16.2; Cesaro *et al.* 1982). The apparent pK_a of D-galacturonic acid is 3.52. Thus, in low-acid foods the carboxyl groups of pectin will be completely ionized. However, in acid foods such as fruits or pickled vegetables, a large fraction of the carboxyl groups will be protonated and unavailable for ionic cross-linking by cations.

The ability of pectin to interact with metal ions, particularly calcium ions, is a major factor in its behavior in processed foods. Kohn

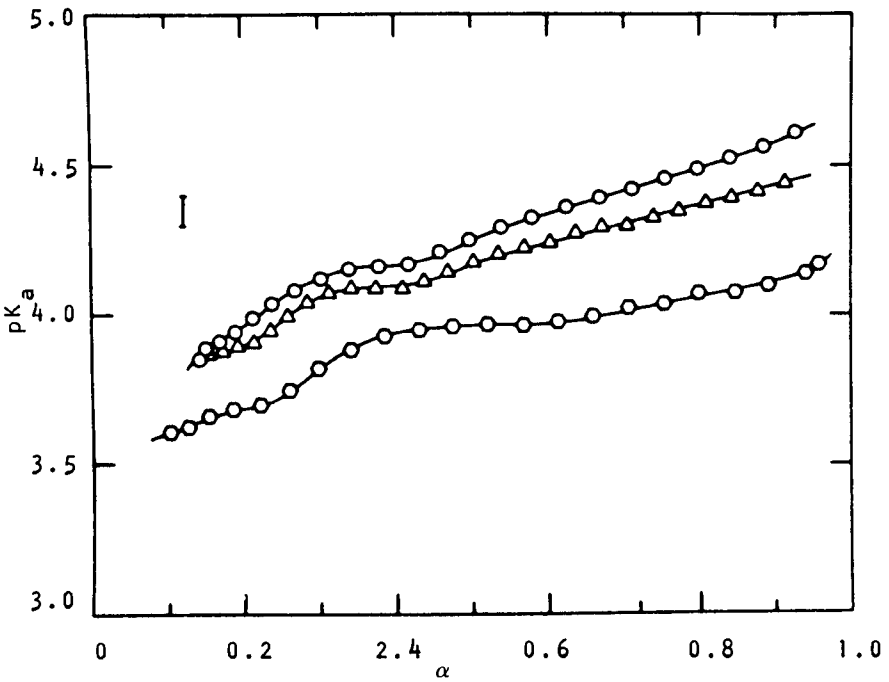

FIG. 16.2. Dependence of the apparent pK_a on the fraction of ionized carboxyl groups (α) for an aqueous solution of pectic acid titrated with 5.0×10^{-3} (○); 6.3×10^{-3} (△); 2.13×10^{-2} (○) equivalent /liter, respectively. The experimental pK_a value of D-galacturonic acid is 3.52. The vertical bar represents the estimated error on the absolute value of pK_a.
From Cesaro et al. (1982).

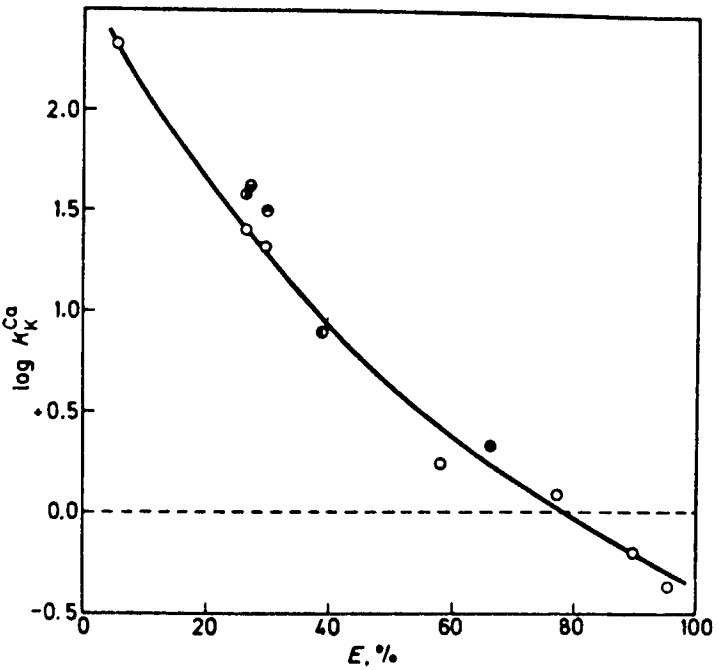

FIG. 16.3. Dependence of selectivity coefficient (K_K^{Ca}) of Ca^{2+}–K^+ exchange reaction in pectin on its degree of esterification (E).
From Kohn (1975).

and co-workers have done extensive studies of calcium binding to the potassium salt of polygalacturonans (Kohn 1975). Their major findings are that the strength of calcium binding increases as the degree of esterification decreases. Kohn (1975) defined a selectivity coefficient (K_K^{Ca}) to express the relative strength of binding of calcium ions to potassium ions in ionic polysaccharides. Figure 16.3 shows that calcium binding is not favored over potassium ion binding when the degree of esterification is about 80%, but with less than 10% esterification, the selectivity of calcium is favored by a factor of over 100. Kohn *et al.* (1975) have determined selectivity coefficients for several metal ions in cross-linked polypectate. Table 16.5 shows that Pb^{2+} and Cu^{2+} bind much more strongly than calcium. The effect of oligomer size on calcium ion activity using mannuronate, guluronate, and galacturonate oligomers has also been studied (Fig. 16.4). The calcium activity in mannuronate solutions decreases as oligomer size increases as a smooth function. However, a discontinuity in the decrease of the calcium ion activity occurs with both guluronate and

TABLE 16.5. SELECTIVITY COEFFICIENTS (K_K^{Me}) FOR THE EXCHANGE OF K^+ IONS WITH DIVALENT METALS (Me) IN CROSS-LINKED POLYPECTATE[a]

Metal	Selectivity coefficient
Mg	26
Ca	121
Sr	120
Co	241
Pb	2580
Cu	3300

From Kohn et al. (1976).
[a] $\mu = 0.15$ and the mole fraction of metal ion = 0.5.

galacturonate. The point at which the discontinuity occurs has been interpreted by Grant *et al.* (1973) as the point at which cooperative binding of calcium ions into an "egg box" structure begins to occur (Fig. 16.5). This corresponds to a degree of polymerization (DP) of 14 for galacturonate and about 20 for guluronate chains.

Investigations of changes in the circular dichroism (CD) spectrum when calcium displaces sodium ions in polypectate indicate that, in the presence of excess monovalent ion (0.5 *M* monovalent ion/6 m*M* calcium ion), polypectate molecules form dimers in an egg box conformation, with 50% of the carboxyl groups neutralized by calcium ion (Morris *et al.* 1982). However, with more than 40% random esterification of carboxyl groups, dimerization of pectin chains does not occur. If monovalent ions are not present, the dimers will further aggregate into a gel network without changes in conformation so that the carboxyl groups will be fully occupied by calcium ions.

Powell *et al.* (1982) demonstrated the importance of calcium cross-links in calcium pectate gels by showing that the strength of the gels decreased as short blocks of polygalacturonate residues (DP≈25) were mixed with the larger residues. It was concluded that the deesterified blocks formed cross-links with the larger polypectate molecules, but because of their small size, they could not form a strong gel network. If the DP ≈ 25 blocks were highly esterified (84%), gel strength was not affected. Esterified blocks would not cross-link with the large polypectate chains and, as a result, had little effect on the gel structure.

One aspect of ion binding to pectin that has not received sufficient attention is the binding of monovalent ions. It is commonly observed that polypectate solutions will gel when sodium chloride is added (McFeeters *et al.* 1980), although higher concentrations of sodium ions than calcium ions are required. With more than 50% methylation,

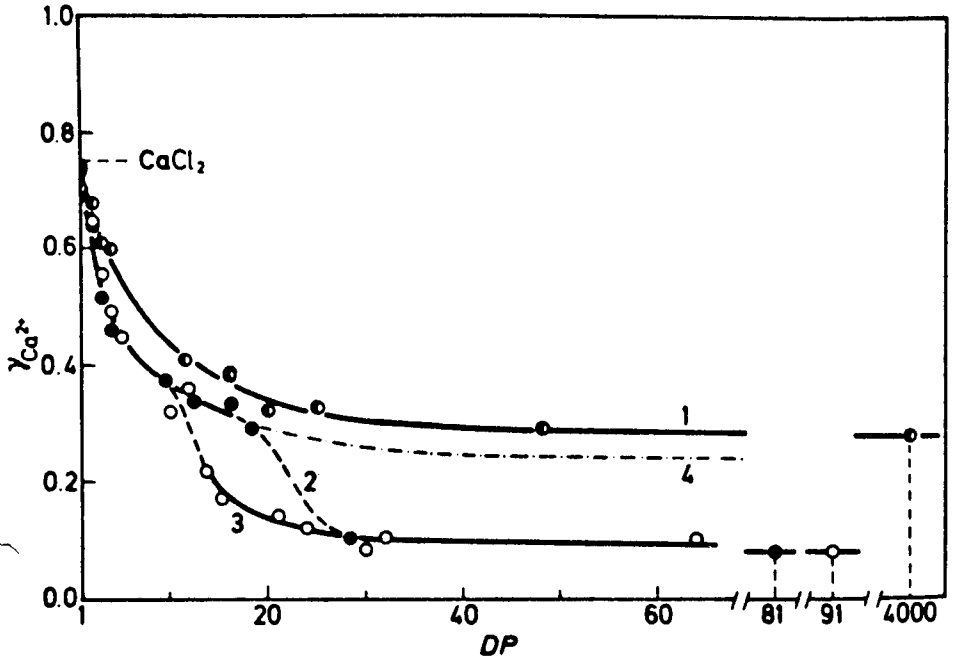

FIG. 16.4. Activity coefficient (γCa^{2+}) in solutions of calcium oligo- and polyuronates as a function of the degree of polymerization (DP): 1, mannuronate; 2, guluronate; 3, galacturonate; 4, theoretical (γCa^{2+}) values in solutions of calcium polyguluronate and calcium polygalacturonate.
From Kohn (1975).

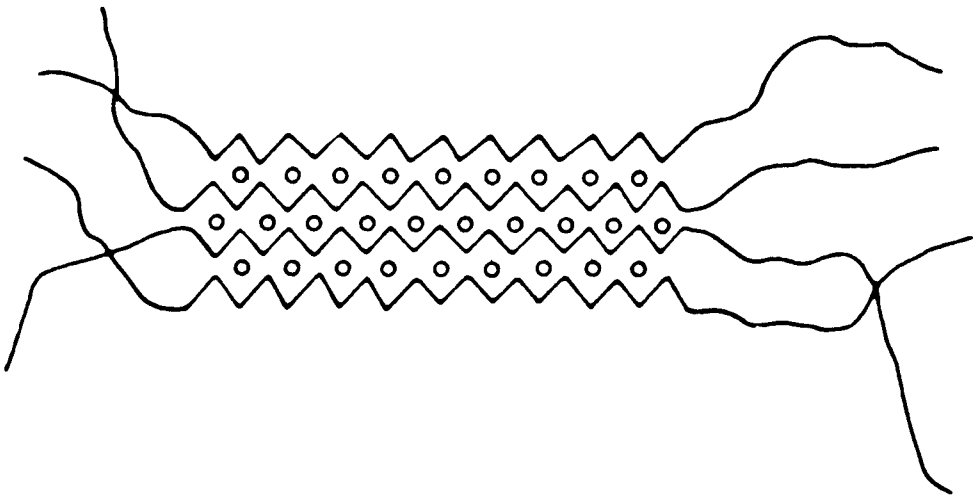

FIG. 16.5. Egg box structure formed by calcium cross-linking of polypectate chains.
From Grant et al. (1973).

pectin will not gel even in very high salt concentrations. The gelation model which has been developed for calcium and other divalent cations does not explain gelation by monovalent ions, since there is not the possibility for divalent cross-linkages.

Seale *et al.* (1982) have measured CD spectral changes and viscosity changes in alginate solutions containing various monovalent cations. Sodium ions were unique because changes in the CD spectrum were similar to those that occurred when egg box binding occurred with calcium. Furthermore, at high alginate concentrations, higher sodium ion concentrations (0.5 *M*) gave higher viscosity solutions than low sodium concentrations (0.05 *M*). In the case of potassium ion, which did not show an egg box spectrum, there was no difference in the viscosity with low and high potassium concentrations. The results were interpreted to indicate cooperative egg box binding of Na^+ between poly-L-guluronate chain sequences, which were analogous to calcium ions but considerably weaker. A model is still needed to explain the way in which a monovalent ion can cross-link polymer chains.

PECTIN STRUCTURE IN CELL WALLS

Detailed structures have not been determined for the pectic polysaccharides of any plant. The greatest amount of structural information has been obtained on pectic substances from suspension-cultured sycamore cells and apples. Recently, Stevens and Selvendran (1984) have also done an extensive analysis of pectic substances from cabbage.

Table 16.1 shows that the polysaccharide fractions, which contain a large proportion of galacturonic acid from primary cell walls of sycamore, are rhamnogalacturonan I, homogalacturonan, and rhamnogalacturonan II. McNeil *et al.* (1984) have reviewed recent work on the structures of these complex polysaccharides. Rhamnogalacturonan I contains galacturonic acid, rhamnose, galactose, arabinose, and occasionally fucose residues and has a high molecular weight with an estimated DP of about 2000 (McNeil *et al.* 1984). The main polymer chain contains alternating 1,4-linked galacturonic acid and rhamnose 1,2-linked residues. About half of the rhamnose units are branched at the 4 position with several different side chains, which average about seven sugar residues in length. The presence of homogalacturonan in the cell wall is inferred from the fact that upon treatment with an endopolygalacturonase, mono-, di-, and trigalacturonic acids are released. The sizes of the homogalacturonan chains are not

known, but partial acid hydrolysis of sycamore cell walls has resulted in the release of polymers with 15 or more residues.

Rhamnogalacturonan II is also released from cell walls by endopolygalacturonase treatment. The isolated polysaccharide contains only about 60 residues, but it is the most complex polysaccharide characterized from the cell walls. Galacturonic acid and rhamnose are the most abundant sugars, but it also contains galactose, arabinose, apiose, 2-*O*-methylfucose, glucuronic acid, 2-*O*-methylxylose, fucose, and aceric acid, the first branched-chain acidic sugar ever found in a polysaccharide.

Pectic substances from apple cell walls have been the most extensively studied pectins from fruits and vegetables. Knee (1973) and Knee *et al.* (1975) extracted a methylated polygalacturonate fraction and a branched, methylated polygalacturonate fraction containing side chains of arabinose and galactose from apple cell walls. DeVries *et al.* (1981) devised an extraction procedure which resulted in removal of 35 and 46% of the galacturonic acid from the alcohol-insoluble solids of unripe and ripe apples, respectively. The extracted pectins were subjected to extensive fractionation by DEAE–cellulose chromatography, gel filtration, and degradation by purified enzymes (DeVries *et al.* 1981, 1982, 1983A). The main conclusion of this work is that apple pectins consist of "hairy" regions with a high degree of neutral sugar branching and "smooth" regions, which are homogalacturonan sections with little or no branching. The largest portion (>90%) of the extracted pectin appears to be homogalacturonan. The hairy sections contain arabinogalactan side chains with a DP of about 25 and xylogalactan side chains with xylose attached to the galacturonan main chain. Esterification of the hairy regions of apple pectin is almost 100%, while the homogalacturonan regions have an average esterification near 70% (DeVries *et al.* 1983B). The pectic substances isolated from apple juice were found to have an average molecular weight of 95,000 with 92% of the galacturonic acid in the homogalacturonan areas and the remainder in the hairy regions (Rouau and Thibault 1984).

RECENT STUDIES OF PECTIC ENZYMES

There are several groups of enzymes produced by higher plants, molds, yeasts, and bacteria which specifically attack pectins. These include pectinesterases, which hydrolyze methyl ester groups, polygalacturonases, which hydrolyze the glycosidic bonds between galacturonic acid residues, and pectin or pectate lyases, which split poly-

galacturonan chains by a β-elimination reaction that leaves a double bond in the 4,5 position of the nonreducing terminal residue. More detailed discussions of the classification and properties of these enzymes have been published (Pilnik and Rombouts 1978; Rexova-Benkova and Markovic 1976).

The effects of pectic enzymes in food products may be desirable or detrimental, depending upon what is to be accomplished. Here, I shall briefly mention recent developments which may be important as we try to better understand and control the action of these enzymes in foods. Most interesting from a processing point of view has been the recognition that polygalacturonases, which hydrolyze the main chain of pectin, commonly are least heat stable at 50°–70°C and more stable at higher temperatures. Archer and Fielding (1975) first described such behavior for polygalacturonase from *Sclerotinia fructigena* (Fig. 16.6). Recent studies have demonstrated similar high-temperature stability for polygalacturonases from several species of *Rhizopus* and

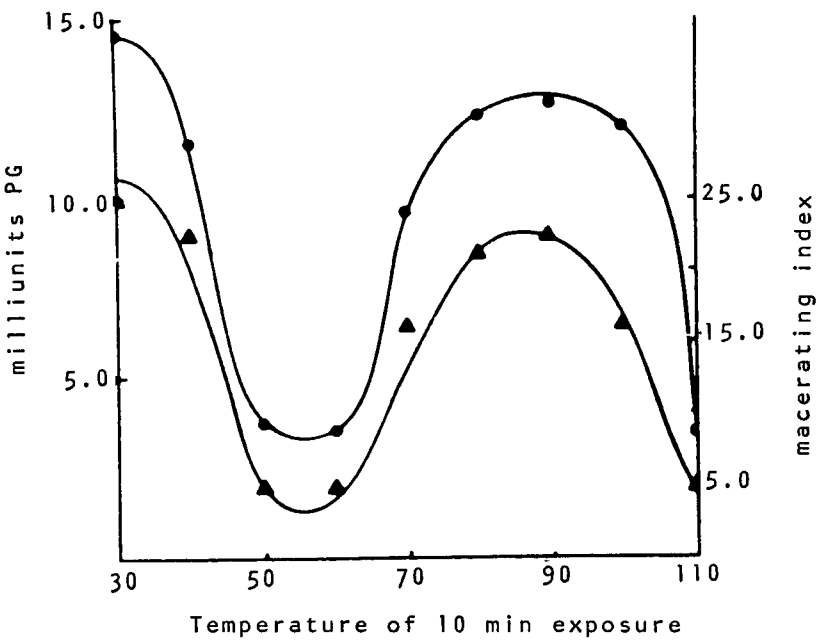

FIG. 16.6. Temperature-inactivation pattern for polygalacturonase isoenzyme from *Sclerotinia fructigena*. Residual PG activity, ●; residual macerating activity (cucumber), ▲.
From Archer and Fielding (1975).

Mucor (Harris and Dennis 1982), suggesting that bimodal heat stability may be common for fungal polygalacturonases.

It has been known for a number of years that plant pectinesterases remove methyl groups from pectin in blocks rather than by random attack, as occurs with acid or base hydrolysis of methyl esters (Rexova-Benkova and Markovic, 1976). However, Ishii *et al.* (1979) and Baron *et al.* (1980) suggested that pectinesterases from *Aspergillus japonicus* and *Aspergillus niger* removed methyl groups from pectin in a random manner. Kohn *et al.* (1983) have confirmed a random attack mechanism by a pectinesterase from *Aspergillus foetidus*. They demonstrated that pectin partially deesterified by this enzyme exhibits calcium binding properties that are nearly the same as alkali-deesterified pectin and very different from the binding characteristics of pectin treated with tomato or alfalfa pectinesterase.

The stability properties of pectinesterases from oranges and cucumbers have been the subject of recent investigations. Three forms of pectinesterase with very different stability properties have been isolated from navel oranges (Versteeg *et al.* 1980). A high-molecular-weight form of pectinesterase (MW 54,000) constituting only about 5% of the total enzyme activity in the orange requires temperatures of 90°C or higher for rapid inactivation. If it is not inactivated, it causes cloud loss in orange juice. Therefore, pasteurization processes must be designed to assure inactivation of the high-molecular-weight form of pectinesterase. The first case of reactivation of a pectinesterase has been found in cucumber slices (McFeeters *et al.* 1985). When fresh cucumber slices were blanched for 3 min at 81°C, enzyme activity could not be detected. However, when the blanched slices were stored in a pH 3.7 brine containing 0.6% acetic acid, 2.5% NaCl, and 200 ppm SO_2, about 20% of the activity present in the fresh tissue was regained during the first month of storage.

ROLE OF PECTIN IN FRUIT AND VEGETABLE TEXTURE

The importance of pectin to the strength and firmness of fruit and vegetable tissues is indicated by the fact that if tissues are treated with various polysaccharide-degrading enzymes, only polygalacturonases are capable, by themselves, of causing extensive softening and cell separation (Mussell and Morre 1969; Wallner and Bloom 1977). Work has been done to use endo-splitting polygalacturonases to produce enzymatically disintegrated vegetables for use in baby foods and vegetable juice drinks (Zetelaki-Horvath and Gatai 1977). Though there

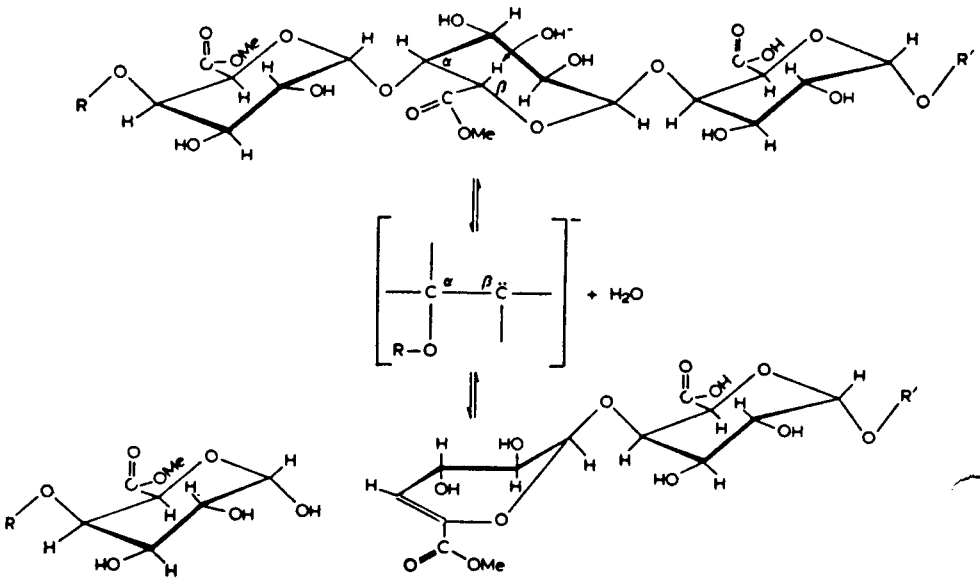

FIG. 16.7. Base-catalyzed β-elimination of esterified galacturonic acid residues. *From Keijbets and Pilnik (1974).*

is no doubt that pectic substances are very important to the texture of fruits and vegetables, determination of the specific ways in which changes in pectins affect texture modifications in foods is quite difficult. Plant cell walls contain a variety of polysaccharides as well as some proteins, so there are almost always problems in assigning relationships between textural changes and specific structural changes.

Albersheim *et al.* (1960) found that pectin can be degraded by a β-elimination reaction in neutral and alkaline solutions (Fig. 16.7). The elimination reaction requires a methylated carboxyl group which assists removal of the proton from C-5 of the galacturonic acid residue. Keijbets and Pilnik (1974) found that β-elimination could occur when slightly acidic (pH 6.1) solutions of pectin were boiled for 30 min. Elimination was enhanced by the presence of both cations (Ca^{2+}, Mg^{2+}) and organic anions (citrate, malate, phytate) compared to K^+ and Cl^-. Similar changes in pectin were also observed when cell walls isolated from potatoes were boiled in pH 6.1 buffer (Keijbets *et al.* 1976). However, significant β-elimination was not observed when isolated cell walls from either potatoes or water chestnuts were heated in deionized water for shorter periods of time ($\leq$5 min) at 90°–110°C (Loh and Breene 1982). The heat treatments used were sufficient to cause considerable loss in fracturability of both potato and water chestnut tis-

sues. Though the pH, heating conditions, and salt concentrations in canned vegetables are such that β-elimination may occur during tissue softening, the reaction has not been directly demonstrated in processed vegetable tissues. For the most part, the specific chemical changes which result in softening of fruit and vegetable tissues during cooking have not been characterized.

The effects of calcium ion on tissue texture have been investigated in a variety of products and process conditions. There are many cases in which addition of calcium either increases the firmness of tissues or prevents loss of firmness. Perhaps the most common observation has been that fruit and vegetable firmness is improved by calcium addition after a mild blanch treatment. This type of firming has been found for frozen and canned snap beans, cauliflower, potatoes, tomatoes, cherries, apples (Van Buren 1979 and references), and carrots (Lee *et al.* 1979). The explanation of the firming effect is that blanching at 50°–80°C activates pectinesterase activity, pectin demethylation occurs, and calcium ions cross-link the demethylated pectin, which increases tissue firmness (Bartolome and Hoff 1972; Hoogzand and Doesburg 1961; Wiley and Lee 1970). However, Moledina *et al.* (1981) concluded that the firming effect of a precooking heat treatment of potatoes was related more to the increased availability of calcium ion released from the starch than to changes in pectin methylation caused by pectin methylesterase. Recently, a 12% increase in calcium binding by green bean components was observed when the beans were blanched at 71°C instead of 93°C prior to retorting (Van Buren 1980). This was attributed to an increase in the number of calcium binding sites after partial pectin demethylation.

The pectin in cucumber cell walls has been found to undergo extensive demethylation when the unheated fruit are fermented in a 6% salt brine with or without added calcium ion (Tang and McFeeters 1983). This demethylation is accompanied by an increase in mesocarp tissue firmness, which could be caused by pectin cross-linking after demethylation. However, when firmness was lost in the cucumbers without added calcium during storage, there were no significant changes in total pectin, pectin size, or noncellulosic neutral sugars in the isolated cell walls that correlated with firmness loss.

Recent studies in this laboratory on firmness changes and pectin methylation in blanched cucumber slices stored in acid brines (pH≈3.7) show that calcium ion is very effective in maintaining tissue firmness (McFeeters *et al.* 1985). However, the effect of calcium is just as great with high methylation (40–50%) as low methylation (10–20%). Since at the storage pH most free carboxyl groups are also uncharged, conditions, particularly in the samples with high methylation, do not ap-

pear to be conducive to cooperative calcium cross-linking of the pectin to form egg box structures (Grant *et al.* 1973). These results suggest that it may be necessary to look for other mechanisms to explain some of the calcium firming effects that occur in acid products.

CELLULOSE STRUCTURE AND FUNCTION

Cellulose appears to provide the basic structural rigidity to the primary cell walls of all higher plants. The biosynthesis, structure, and degradation of cellulose has been extensively discussed in a recent book (Brown 1982). The polymer consists of linear chains of β-1,4-linked glucose residues which form both intra- and interchain hydrogen bonds to produce highly insoluble cellulose fibers (Fig. 16.8). In the cell walls of higher plants, cellulose molecules are arranged in microfibrils about 10 nm wide and 5 nm thick (Preston 1974). There are crystalline and amorphous regions within the microfibrils. The crystalline regions may be as long as 80–120 nm. Based upon X-ray diffraction studies of cellulose from the algae Valonia, it is believed that within the microfibrils, cellulose molecules have a parallel arrangement (Gardner and Blackwell 1974). Cellulose molecules have been found to contain from about 1000 (Thornber and Northcote 1962) (160,000 MW) to 44,000 glucose units (7,000,000 MW) for a high-molecular-weight cellulose fraction from Valonia (Palma *et al.* 1976).

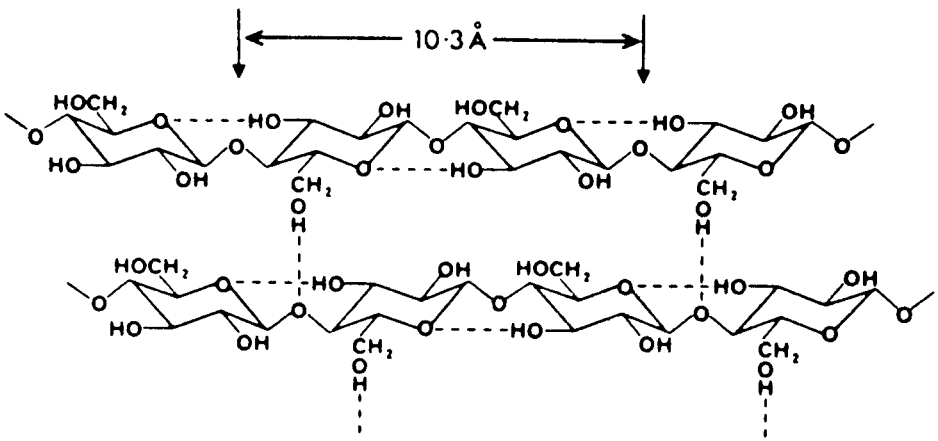

FIG. 16.8. The structural features of the β-(→4)-linked D-glucan chains of cellulose.
Adapted from Selvendran (1983).

Cellulose chains have an extended ribbon conformation, with two glucose residues repeating every 10.3 Å. Therefore, the largest cellulose molecules will be over 20 μm long. Considering the relative length of crystalline cellulose regions and the length of individual molecules, a single molecule may pass through many crystalline regions.

Most research on cellulose structure has been done using systems such as Valonia and cotton fibers, which produce large amounts of reasonably pure cellulose. Information on molecular size, proportion of crystalline and amorphous cellulose, and detailed microfibril structure has not been obtained on cellulose from fruit and vegetable tissues.

Approximately 20–60% of the cell wall polysaccharides from fruits and vegetables is cellulose. Generally, this cellulose contains 5–20% of sugars other than glucose, including xylose, arabinose, galactose, and uronic acid (Voragen *et al.* 1983; Selvendran 1983). It is not clear whether these sugars are covalently bound to cellulose or whether there are strong noncovalent attachments. O'Neill and Selvendran (1982) have reported that hydroxyproline-rich glycoproteins may be covalently cross-linked to cellulose through phenolic compounds.

Cellulose is much less reactive in foods than pectin, so there have been few investigations of changes in cellulose during processing and storage of foods. One area that has received some attention has been enzymatic degradation of cellulose by cellulases. The main interest in this research has been the development of economic processes for conversion of cellulose to ethanol via glucose (Wilke 1975; Gaden *et al.* 1976). Efforts to utilize cellulases in food processing have been primarily directed toward improving the extraction of cellular components. Examples include the isolation of starch (Toyama 1969) and extraction of olive oil (Fantozzi *et al.* 1977; Leone *et al.* 1977). There have also been attempts to use cellulases in the production of vegetable purees (Toyama 1969; Ghose and Pathak 1973).

Cellulase, which degrades carboxymethylcellulose but not crystalline cellulose, has been found to be present in tomatoes (Pharr and Dickinson 1973), peaches (Hinton and Pressey 1974), and mangoes (Roe and Bruemmer 1981). The cellulase activity increased during ripening in each of these fruits. However, the role of this cellulase activity in causing texture changes in the fruit has not been determined.

Degradation by reactive oxygen species is a reaction which cellulose shares with most, if not all, polysaccharides. Kon and Schwimmer (1977) added xanthine oxidase to polysaccharide solutions to generate superoxide anion and hydrogen peroxide, and then measured viscosity changes. Each polysaccharide tested, including methylcel-

lulose, starch, pectin, and guar gum, showed a 30–50% decline in viscosity, which suggested that degradation of the polysaccharide chains had occurred. The effect of inhibitors on the reaction indicated that degradation was caused by hydroxyl radical and singlet oxygen. They suggested that the potential exists for generation of reactive forms of oxygen in foods which may result in texture changes. This remains to be demonstrated in food systems. There have been recent investigations of possible mechanisms by which hydroxyl radicals can attack dextrans and pectins (Gilbert *et al.* 1984).

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