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THE CHEMISTRY OF TEXTURAL CHANGES IN FRUIT DURING STORAGE

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ABSTRACT

Cells in plant tissue are surrounded by cell walls which are comparatively rigid and give mechanical support to the tissue. The walls of pome fruits, strawberry and tomato contain a high proportion of galacturonic acid, galactose and arabinose residues which are typical of pectic polysaccharides. The bonding between the polymers in the middle lamella, the region of the wall between adjacent cells, is thought to be ionic in nature involving Ca^{2+} and carboxyl groups of the pectic polysaccharides. Structural changes occur in the middle lamella and primary cell wall during ripening which lead to cell separation and softening of the tissue.

Softening is characterised by an increase in the concentration of soluble pectic polysaccharide. In apple, the molecular weight of this fraction remains unchanged and endo-polygalacturonase (endo-PG), a random cleavage enzyme, is absent. Exo-polygalacturonase (exo-PG), a terminal cleavage enzyme, is present and approximately 10% of the galacturonic acid residues of the cell wall are lost during ripening. In other fruits, such as pear and peach, both exo- and endo-PG activities develop and the molecular weight of the soluble pectic polysaccharide decreases. It is concluded that the softening observed in ripening fruits derives from the synthesis and transport to the cell wall of wall degrading glycosidases.

INTRODUCTION

Softening of the fleshy tissues of fruits is one of the most important changes occurring during storage and has a major influence on customer acceptability. The texture of living plant tissue is affected by its cellular anatomy, the water relations of the cells and the composition of the cell walls. Fruit cells retain their normal osmotic

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properties during ripening (Simon, 1977) and there is probably little loss of turgor pressure although this may be affected by dehydration if fruit is kept in a low humidity. Turgor pressure probably provides a driving force for cell separation following structural changes in the middle lamella and primary cell wall.

CELL WALL STRUCTURE

Plant primary cell walls

The primary wall consists of cellulose microfibrils embedded in a matrix of pectic and hemicellulosic polysaccharides and hydroxyproline-rich glycoprotein (Northcote, 1972). The model structures established by Albersheim and his co-workers (Talmadge *et al.*, 1973; Bauer *et al.*, 1973 and Keegstra *et al.*, 1973) for rhamnogalacturonan with its side chains of galactose and arabinose residues (Fig. 1) and for xyloglucan have gained general acceptance. However, the proposed

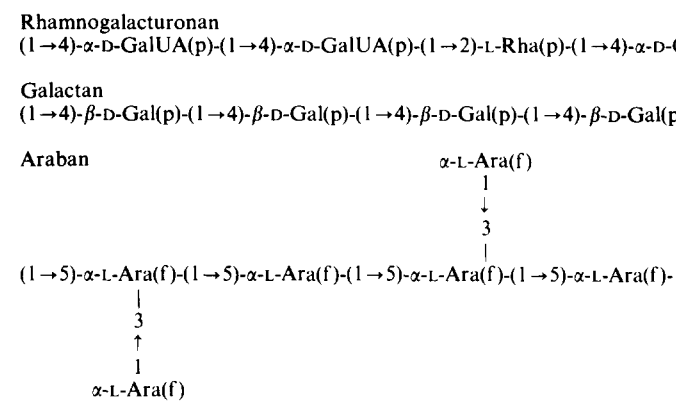

Fig. 1. General features of pectic polysaccharides. Standard abbreviations are used to represent the monosaccharides and (p) and (f) denote pyranose and furanose forms, respectively.

covalent linkages between these polymers and others in the cell wall have proved more controversial. Keegstra *et al.* (1973) presented evidence for a linkage of xyloglucan to rhamnogalacturonan through a galactose chain, and of rhamnogalacturonan to protein through arabino-galactan linked to serine. The existence of these linkages has not been substantiated and other authors (for example, Monro *et al.*, 1976) have preferred structures based on non-covalent bonding.

Water is an important constituent of the cell wall (Northcote, 1972). The quantity of water within the wall matrix can be controlled to some extent by the proportions of the polymers as pectic polysaccharides are able to bind much more water than the hemicelluloses (Cook & Stoddart, 1973). Some of the water associated with the

pectic polysaccharides is tightly bound and is required for the maintenance of the conformation of the polyuronide chains. Water also acts as a solvent within the wall for the presence and transport of salts and low molecular weight organic compounds, and provides a suitable environment for the function of enzymes such as the glycosidases which hydrolyse cell wall polymers.

The galacturonan backbone of pectic polysaccharides exists as an extended ribbon-like chain with a threefold screw axis (Rees & Wight, 1971). Rhamnose residues occur within the chain and at these points the chain is bent or kinked (Rees & Wight, 1971). The rhamnose residues are the points of attachment for side chains of galactose and arabinose (McNeil *et al.*, 1980). The gel is the state most typical for polysaccharides in biological systems, the polymer chains usually forming an interconnecting network which gives rise to characteristic texture and properties (Rees, 1969). Molecules of water are held within the interstices of the network. The galacturonan molecules of the plant cell wall are thought to form an interconnecting network through the formation of non-covalent bonds at regions of chain association, known as junction zones. Galactose residues occur as β-1,4-linked linear polymers whilst the arabinose residues occur as branched arabans. The arabinofuranose residues form a hydrophilic network so that these molecules can also hold water within the gel structures formed by the galacturonan (Northcote, 1972).

Analyses of cell wall pectic polysaccharides using chemical (Cook & Stoddart, 1973) and enzymic methods (Talmadge *et al.*, 1973; Knee *et al.*, 1975; McNeil *et al.*, 1980) indicate that the neutral sugar side chains occur in blocks interspersed in the rhamnogalacturonan. The pectic polysaccharides of the primary wall are thought to be more highly substituted than the polymers of the middle lamella (Knee *et al.*, 1975). The rhamnose residues which cause kinking in the galacturonan would prevent alignment of chains, whilst regular packing could be hindered by the side chains (Rees, 1969). The pectic polysaccharides of the primary wall might therefore be expected to form a less extensive gel network than the middle lamella polymers.

Whereas genes control the assembly of peptide chains by an accurate template mechanism, the synthesis of the matrix polysaccharides of the cell wall is catalysed by a large number of glycosyl transferases coded in the genetic material of the cell (Northcote, 1972); the polysaccharides are thus secondary gene products assembled by the concerted action of multiglycosyl transferase systems. Little is known about the regulation of matrix polysaccharide biosynthesis (Delmer, 1977) but, by analogy with the synthesis of the carbohydrate moiety of glycoproteins, control of polymer size and composition could be mediated by a number of factors including the concentration and structural features of donor and acceptor molecules and the concentration and specificity of the transferases (Pazur & Aronson, 1973). Polysaccharides are polydisperse in nature. For example, the pectic polysaccharides, although essentially similar in structure, may have different proportions and distributions of sugar residues and also display variation in the

frequency of branch points (Reid & Wilkie, 1969). Polysaccharides are also polymolecular, showing a range of molecular weights (Reid & Wilkie, 1969), although they generally tend towards an upper limit of molecular weight which is often very high (Cook & Stoddart, 1973).

Structure of the middle lamella

Histological studies of ripening fruit reveal extensive cell separation and it is natural to suppose that changes in the intercellular matrix, the middle lamella, are responsible for this. Electron microscope studies of the cell walls of apple and pear during ripening support this hypothesis (Ben-Arie *et al.*, 1979). Traditionally, the middle lamella has been thought to be rich in pectic polysaccharides and this has been confirmed by use of ferric hydroxamate staining prior to electron microscopy (Albersheim & Killias, 1963). The observation that cells of non-woody plants can be dissociated using chelating agents (Ginsburg, 1961; Letham, 1962; Linehan & Hughes, 1969) suggests that cohesion of the middle lamella depends on ionic rather than covalent bonds. Divalent cations, especially calcium, are the obvious candidates, but, as Rees (1969) pointed out, single calcium ions would only form weak bonds between isolated carboxyl groups. More stable bonding occurs by co-operative effects when sequences of uronic acids lie parallel and each pair of residues encloses a calcium ion; the so-called 'egg box' junction zones (Grant *et al.*, 1973; Morris, 1980).

Fruit cell walls

Apart from the vascular tissue and specialised cells, such as the stone cells in pears, the cell walls of the fleshy parts of most fruits are unlignified. They also contain a low proportion of hydroxyproline-rich protein and small amounts of xylose and mannose residues which are characteristic of hemicellulosic polysaccharides; on the other hand, they contain a high proportion of galacturonic acid, galactose and arabinose residues which are typical of pectic polysaccharides (Knee *et al.*, 1975).

Purified glycosidases, mainly from fungal sources, have been used in detailed structural studies of apple fruit cell walls (Knee *et al.*, 1975). The pectic polysaccharides in the walls are (a) a simply substituted methyl esterified galacturonan which is probably localised in the middle lamella and (b) a branched methyl esterified rhamnogalacturonan probably concentrated in the primary wall. Branched araban chains are covalently linked to the rhamnose residues of the rhamnogalacturonan, and linear galactan chains are attached to galacturonosyl residues of the polymer (Barrett & Northcote, 1965). The branched rhamnogalacturonan shares most of the features of the rhamnogalacturonan of sycamore suspension culture cell walls (Talmadge *et al.*, 1973) and forms the main constituent of the primary wall.

Subsequent chemical studies showed that the simply substituted galacturonan in

apple cell walls could be solubilised by partial methylation of its free carboxyl groups with diazomethane (Knee, 1978*a*). Diazomethylation or treatment with the chelating agent, sodium polyphosphate, also caused loss of cell to cell cohesion in ethanol-extracted tissue (Knee, 1978*a*). This suggested that at least some of the 20 % of non-esterified carboxyl groups in the cell wall are important in maintaining cell cohesion by cross linkage through divalent metal ions.

Single cross links are weak and a stable structure would require the alignment of non-esterified sequences of uronic acid in otherwise esterified chains; as yet nothing is known about the distribution of methyl ester groups in the galacturonan.

Chromatographic purification of an alkaline extract of apple cell walls (Knee, 1973*a*) yielded a polysaccharide of similar gross composition to the xyloglucan of sycamore cell walls extensively studied by Bauer *et al.* (1973). Although xylose and glucose residues are often present in pectic polysaccharide fractions obtained by enzymic (Knee *et al.*, 1975) or chemical (Knee, 1978*a*) degradation of apple fruit cell walls, there is no direct evidence of linkage between the hemicellulosic and pectic polysaccharides.

ENZYMES ASSOCIATED WITH CELL WALL DEGRADATION

A number of glycosidases have been implicated in the degradation of cell wall polysaccharides, together with an esterase, pectin esterase, which catalyses de-esterification of methyl esterified pectic polysaccharide (Table 1). β-Galactosidase and exo-polygalacturonase (exo-PG) are terminal cleavage enzymes, releasing monosaccharides from the non-reducing end of the substrate. Endo-polygalacturonase (endo-PG) and endo-β-1,4-glucanase are random cleavage enzymes. The pH optimum of the exo-PG and endo-PG, determined in *in vitro* assay, is in the range 4·0–5·5, whilst for fruit pectinesterases, assayed with NaCl (0·05–0·20M), the

TABLE 1

MOLECULAR WEIGHTS OF ENZYMES ASSOCIATED WITH THE HYDROLYSIS OF CELL WALL POLYMERS OF RIPENING FRUITS

Enzyme	*Molecular weight*[a]				
	Apple	*Pear*	*Peach*	*Tomato*	*Avocado*
Exo-PG	58000	63000	68000		
Endo-PG		160000	41000	44000; 84000[b]	
β-Galactosidase	46000			63000	
Endo-β-1,4-glucanase					49000
Pectinesterase	27000			23700–35500[c]	

[a] Data for enzymes of apple, Bartley (1978) and unpublished and Miyairi *et al.* (1975); pear, Pressey & Avants (1976) and Pressey (personal communication); peach, Pressey & Avants (1973*a*); tomato, Pressey & Avants (1972. 1973*b*) and Wallner & Walker (1975); avocado, Awad & Lewis (1980).

[b] Major endo-PG has molecular weight 44000.

[c] Four pectinesterases characterised, molecular weights 23700, 24300, 27000 and 35500.

pH optimum is usually 7·0–8·5 (Rexova-Benkova & Markovic, 1976). Pectinesterase is also activated by Ca^{2+}. For example, the pH optimum of the orange enzyme changes to 5·0–8·0 in the presence of 0·05M $CaCl_2$ (MacDonnell *et al.*, 1945).

Assay of endo-PG and exo-PG with galacturonan and methyl esterified galacturonan substrates (Pressey & Avants, 1973*a*; Bartley, 1978) indicates that the de-esterified substrate is more rapidly hydrolysed and suggests that pectinesterase may influence the rate of hydrolysis of cell wall polymers by the glycosidases. However, in apple, in contrast to pear, peach and tomato, the degree of esterification of the pectic polysaccharides does not decline during ripening but remains essentially unchanged, and this may question the postulated rôle of pectinesterase in this fruit (Doesburg, 1965; Knee, 1978*a*). The pH of the fruit cell walls is not known, although, from the pH optima of the glycosidases, it might be expected to be below 7·0. Partially purified PG's can release polymers of uronic acid from fruit cell wall preparations in *in vitro* incubations at pH 4·0–4·5 (Pressey & Avants, 1973*a*, 1976; Bartley, 1978).

It is notable that, of the enzymes associated with cell wall degradation in ripening fruits (see Table 1), only the endo-PG of pear has a molecular weight (MW) in excess of 100,000. A comparison of the hydrolysis of apple fruit cell wall preparations using a number of endo-PG's (MW 37-200,000) and also α-L- arabinofuranosidases (MW 40-350,000) suggested that enzymes with a molecular weight greater than 100,000 are excluded from the wall and cannot degrade it, irrespective of their activities with soluble substrates (Knee *et al.*, 1975). Carpita *et al.* (1979), working with living plant cells including cultured cells of sycamore maple, calculated that globular proteins having a molecular weight greater than 17,000 cannot diffuse freely through the cell wall.

WALL DEGRADATION DURING FRUIT RIPENING

Cellulose

Crystalline α-cellulose is very resistant to enzymic attack and can only be degraded by the combined operation of a number of glycosidases, including the endo-glucanases C_1 and C_x (Rees, 1977). The C_1 glucanase, which is thought to initiate degradation of the polymer, has not been detected in fruit and explains why degradation of cellulose has not been observed during fruit ripening.

Hemicelluloses

The small quantities of monomers characteristic of the hemicelluloses, xylose, glucose and mannose, do not decline during the ripening of apples (Bartley, 1976), strawberries (Neal, 1965), tomatoes (Gross & Wallner, 1979) and pears (Ahmed & Labavitch, 1980). Endo-β-1,4-glucanase is present in a number of fruits including apple (Bartley, unpublished data), pear (Yamaki & Matsuda, 1977), tomato (Pharr

& Dickinson, 1973), peach (Hinton & Pressey, 1974) and avocado (Awad & Lewis, 1980). The activity of the enzyme is normal in the non-ripening *rin* mutant of tomato (Poovaiah & Nukaya, 1979) and this observation, together with the analytical data, suggests that the enzyme does not have a primary rôle in fruit softening.

Loss of galactose residues

The major change in monomeric composition of the cell walls of ripening apples is the loss of about 70% of their galactosyl residue content (Table 2; Knee, 1973*b*).

TABLE 2

ANHYDRO SUGAR COMPOSITION OF POLYSACCHARIDES IN CELL WALL AND SOLUBLE FRACTIONS FROM APPLE AND STRAWBERRY FRUITS

	Apple[a]				*Strawberry*[a]			
	Unripe		*Ripe*		*Unripe*		*Ripe*	
	Cell wall	*Soluble*	*Cell wall*	*Soluble*	*Cell wall*	*Soluble*	*Cell wall*	*Soluble*
Rhamnose	0·11	ND	0·10	ND	0·63	0	0·35	1·39
Arabinose	2·68	0·07	2·00	0·14	3·51	0·15	0·81	3·17
Xylose	0·79	0·01	0·73	0·01	1·03	0·04	1·79	0·30
Mannose	0·32	ND	0·32	ND	0·47	0·02	0·94	0
Galactose	3·78	0·11	0·75	0·14	4·34	0·08	1·56	2·57
Glucose[b]	29·3	0·06	4·44	0·06	16·6	0·28	13·5	3·69
Galacturonic acid	3·90	0·10	2·51	1·00	21·0	4·01	6·74	16·3

[a] Apple results are quoted in mg gram^{-1} fresh weight, strawberry results as mg per fruit (unripe fruit weight 2·41 g, ripe fruit weight 8·68 g).
[b] Results for cell wall glucose include starch.
ND: not determined.

The loss of monomer occurs in the branched pectic polysaccharide fraction (Knee *et al.*, 1975). Similarly in tomatoes (Gross & Wallner, 1979), loss of galactose is the dominant change, but in pears (Ahmed & Labavitch, 1980) and strawberries (Neal, 1965) the change is smaller. However, there is evidence of turnover of galactose residues during growth of strawberry fruits (Knee *et al.*, 1977) and this seems to be a feature of cell expansion in other tissues (Labavitch & Ray, 1974). Apples (Bartley, 1974), tomatoes (Wallner & Walker, 1975) and pears (Yamaki & Matsuda, 1977) all contain β-galactosidase activity. The apple enzyme was shown to be capable of degrading β-1,4-linked galactan (Bartley, 1974) and releasing galactose residues from apple cell wall preparations (Bartley, 1978). Enzyme activity was present at all stages of ripening in the various fruits examined; in the apple, activity associated with the cell wall was detectable at all stages (Bartley, 1977). This leads to the suggestion that hydrolysis of terminal galactosyl residues is a constant feature of some fruit cell walls and that galactose residues decline only when the synthesis of rhamnogalacturonan carrying branches of galactose units fails to make good the losses. The significance of the loss of galactose residues for softening is not clear for in *rin* tomatoes the loss takes place after harvest, when little softening occurs (Gross & Wallner, 1979).

Increase in soluble pectic polysaccharide

It has been known for at least 60 years that the proportion of soluble pectic polysaccharide increases in ripening apples (Carre, 1922). The process is matched by a decline in the insoluble pectic polysaccharide fraction (see Table 2) and it is natural to conclude that a change in part of the insoluble fraction converted it to a soluble form. The ensuing years have seen various interpretations of what that change might be. Joslyn (1962) has reveiwed the older literature but we shall confine ourselves to more recent developments.

The soluble pectic polysaccharide in ripe apples has a low proportion of neutral monomers (Knee, 1973*a*) and this is in keeping with its supposed origin in the middle lamella. Its physical properties, and hence its molecular weight, are comparable with those of the soluble pectic polysaccharide in the unripe fruit (Knee, 1978*a*). The apple is exceptional among fruits in that it appears to lack endo-PG. In other fruits, the enzyme is an obvious means of achieving solubilisation, and the soluble pectic polysaccharide isolated from peaches (Pressey *et al.*, 1971), strawberries (Woodward, 1972) and pears (Knee, 1973*a*) shows progressive depolymerisation with ripening. Endo-PG activity increases in tomatoes and pears during ripening, but activity is not measurable in unripe fruit (Hobson, 1964; Tucker *et al.*, 1980; Knee, Casimir & Bartley, unpublished data). Radioimmunoassay of extracts from green and ripe tomato fruits suggests that the increase in endo-PG activity observed derives from net synthesis of protein (Tucker *et al.*, 1980).

An interesting contrast is provided by different varieties of peach. Freestone varieties possess high activities of both endo-PG and exo-PG; they show extensive softening and a marked rise in soluble pectic polysaccharide on ripening. Clingstone varieties have low activities of endo-PG and show less softening and pectic polysaccharide solubilisation (Pressey & Avants, 1978).

Endo-PG has not been detected in apple, but an exo-PG is present (Bartley, 1978) and this must account for the loss of about 10% of total uronic acid residues during ripening. The enzyme preferentially hydrolyses non-esterified galacturonan, but is capable of degrading apple cell walls to release monomers and some polymeric material. The viscosity of the pectic polysaccharide extracted from the cell wall with sodium polyphosphate declines during ripening (Knee, 1978*a*). This fraction is thought to derive from the middle lamella region of the cell wall and suggests that this is the site of action of exo-PG.

A MODEL OF THE CELL SEPARATION PROCESS

From the data discussed, a model can be proposed for the softening of fruits observed during ripening. It is suggested that the middle lamella is stabilised by junction zones formed by calcium ions and free carboxyl groups in predominantly esterified pectic polysaccharide molecules. Softening is initiated by the transport of

PG to the middle lamella, possibly together with pectinesterase. The apple lacks endo-PG, and the exo-PG would cause a limited degradation of cell wall polymers. In other fruits, endo-PG degrades the soluble pectic polysaccharide, and cell separation and softening are more extensive. The glycosidases display optimal activity at acid pH values; acidification of the cell wall could be brought about by a proton pump located in the plasmalemma. Acidification could also lead to a weakening of cell wall polysaccharide bonding by displacement of calcium ions from junction zones (Soll & Böttger, 1981).

Changes occurring in the abscission zones of plant tissues show analogies with those observed in fruit ripening. Although exo-PG has been prepared from the abscission zones of citrus and bean, endo-PG has not been detected (Riov, 1974; Berger & Reid, 1979). A number of isoenzymes of endo-β-1,4-glucanase have also been assayed. The time course of appearance of the pI 9·5 enzyme and its distribution are consistent with its involvement in abscission (Sexton *et al.*, 1980). It is thought that this enzyme degrades non-cellulosic glucan components of the cell wall.

Secreted proteins are often glycoproteins which undergo post-translational modifications following synthesis of the polypeptide moiety in the endoplasmic reticulum (Chrispeels, 1976). Osborne & Sargent (1976) suggested that hydrolytic enzymes present within the dictyosome vesicles could be secreted to the wall either after fusion of the vesicles with the plasmalemma or by passage of intact vesicles via the desmotubules of the plasmodesmata. Once within the plasmodesmata, any hydrolytic enzymes would be liberated by rupture of the vesicles in the immediate vicinity of the middle lamella. Ben-Arie *et al.* (1979) noted that a characteristic of middle lamella dissolution in ripe apple and pear fruits was the frequent occurrence of vesicles in the vicinity of the plasmodesmata complex. They also noted the persistence of the cell wall–plasmodesmata complex through ripening.

SYNTHESIS OF CELL WALL POLYSACCHARIDES IN RIPENING FRUITS

Most studies of cell wall changes in ripening fruit have concentrated on degradative aspects and the possible rôle of synthetic processes has rarely been considered. A study of polysaccharide metabolism in developing strawberry fruits (Knee *et al.*, 1977) revealed that incorporation of ^{14}C-glucose into wall polymers ceased at the onset of ripening. Strawberry fruits were also labelled with $^{14}CO_2$, 2–6 days after petal fall. A loss of label from insoluble cell wall polymers was observed during ripening of the fruits with a corresponding increase of label in the soluble polymers. This is consistent with the predominance of degradative changes in ripening.

In recent work with apple and pear, tissue discs prepared from the fruits at various stages of ripening were incubated with methyl labelled ^{14}C-methionine and ^{3}H-inositol selected as precursors of cell wall polysaccharides (Knee, 1978*b*). Inositol

incorporation into galacturonan declined sharply during ripening whilst methionine incorporation into methyl ester groups of galacturonan was maintained. This suggested that cell separation might be brought about by methylation of the galacturonan. However, further experiments with pear tissue discs in which uronic acid content was measured together with incorporation of ^{14}C-methionine indicated that the discs synthesised galacturonan and methyl esterified polymer in *in vitro* incubations (Knee, unpublished data). The uronic acid content of whole pears declines continuously during ripening, and the results with discs suggest that polysaccharide synthesis assayed could be a wound injury response to cutting of the tissue (Van Steveninck, 1975).

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