3. FRUIT SOFTENING

Melford A John

Department of Biochemistry, University of the West Indies, Mona, Kingston 7, Jamaica

---

CONTENTS

INTRODUCTION

Many fruits as they ripen, become softer and increasingly susceptible to fungal attack. Optimum quality is often associated with a certain degree of hardness and crispness, beyond which customer appeal diminishes. Development of technology to control this change is therefore of importance to fruit industries. For further information on such technologies and on other ripening changes (see Medlicott and Jeger V.i and Chaplin Chapter V.2).

CAUSE OF SOFTENING

Softening is usually accompanied by breakdown of the plant cell wall. The walls of parenchyma cells in the pulp region of fruits are made up almost entirely of carbohydrate material. Mature unripe fruit cells generally have rigid, well-defined walls whereas those from ripe fruits have soft, diffused walls. Ultrastructural studies have suggested that softening is accompanied by dissolution of the middle lamella region (between cells) which is rich in pectin, resulting in cell separation in tomato (9), strawberry (31), apple (4) and pear (4). This change appears to be brought about by hydrolytic enzymatic activity on the pectic component of the cell wall. Molecules from this fraction characteristically have a long backbone of galacturonic acid in α-1, 4-linkage with side chains containing galactose and/or arabinose attached to rhamnosyl residues (see Fig. 1). Other features of this polymer are regions of high branching, regions of low branching, and areas where the carboxylic acid group of galacturonosyl residues are methoxylated or acetylated.

Once fragmented by enzymes, pectin becomes detached from the rest of the cell wall. Such solubilization has been observed in mango (11,25), tomato (16), strawberry (31), date (47), apple (29), avocado and peach (39). This process together with mobilization of stored reserves of starch accounts for increases in soluble carbohydrates with ripening. Consistent with these observations, are reported decreases in cell-wall content of galacturonic acid, galactose and often arabinose (1,16,17,30,31). There is very little evidence to support significant loss of other cell wall components (hemicellulose, cellulose, hydroxyproline-rich glycoproteins) during softening.

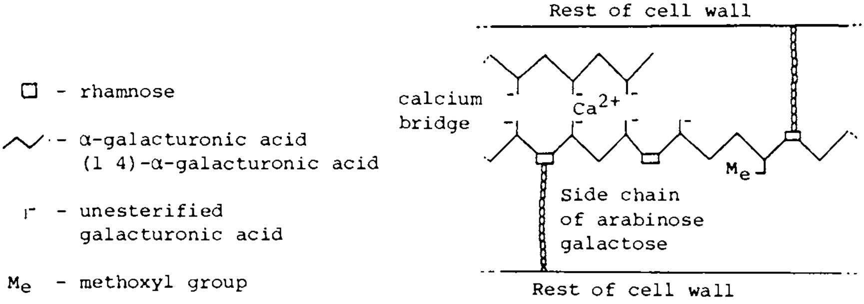

Figure 1. **Structure of pectin and its attachment to the rest of the cell wall.**

ENZYMOLOGY OF SOFTENING

Our present understanding of the mechanism of cell-wall softening is poor. (See Huber (23) for a comprehensive review of cell wall changes and enzyme changes during fruit ripening). One of the main problems is a lack of understanding of the full structure of pectin and how it is associated with the rest of the cell wall. For more detailed coverage of this topic, the reader is referred to recent reviews by John and Dey (28) and by Fry (14). It seems very likely that pectin molecules are covalently attached to the cell wall via their side chains. Thus, solubilization may be effected by internal cleavage of the latter and/or endo-hydrolysis of the rhamnogalacturonan backbone. The former activity requires hydrolysis of endogalactanases or endoarabanases whilst the latter can be effected by endopolygalacturonase.

Endopolygalacturonase (PG)

Endopolygalacturonase has been identified in many fruits (9,19,20,41,42) and possesses the capacity to randomly hydrolyse the rhamnogalacturonan backbone of pectin. However, it is not present in all fruits. After an extensive search, Brinson (8) concluded it was absent from Ngowe mangoes. This was subsequently supported by John (27). However, the enzyme is present in Keitt mangoes (46). Further, in a recent study, Lazan and co-workers (32) reported PG activity in 'Huramanis' and 'Mulgoa' mangoes.

Evidence for the involvement of PG in fruit softening is very convincing. Its activity becomes noticeable two or three days after the onset of ethylene production in tomato (54) and increases with ripening. It can singularly solubilize material from cell walls of unripe tomato (55), pear (2), and peach (40) under in vitro conditions. Using electron microscopy, Crookes and Grierson (9) showed that such activity in tomato can cause dissolution of the middle lamella. Although PG can degrade tomato cell walls in vitro, its action alone does not bring about reductions in cell-wall galactose as occurs during the natural process (55). Thus, it seems likely that cell-wall breakdown is caused by coordinated activity of at least two enzymes in this fruit. In pears, however, the enzyme is able to solubilize arabinose (2). It is becoming apparent that there may be different mechanisms for the solubilization of pectin in different fruits. Certainly, the absence of endo-PG in some fruits such as the apple suggests there are at least two classes of fruits with different mechanisms for cell-wall softening. One involving hydrolysis of the rhamnogalacturonan backbone and the other presumably hydrolysis of the side chains containing neutral sugars. A further complicating factor is variation in the sugar composition of the cell walls of fruits particularly between those belonging to different botanical groups. This has been demonstrated by Gross and Sams (17) who studied the cell-wall sugar composition of seventeen different fruits. They suggested that care should be taken to avoid generalizations about fruit softening. If initial structures are different to start off with, then their mechanism of degradation are likely to vary. This issue revolves around the presently debated question on the universal applicability of cell-wall models. Albersheim (3) suggested that one model may apply to the primary cell-wall of dicotyledonous plants. Presently this contention has not been widely accepted, although it has not been dismissed. Further progress is dependent upon a greater understanding of cell-wall structure and the study of a wider variety of plants. Much of the structure on cell-wall softening has been restricted to work on popular varieties of fruits such as tomato, apple, pear, avocado, mango and peach. These represent a tiny fraction of the entire fruit spectrum and may not necessarily be representative of ripening in general. There is need for widening the number of species studied, and for work of a more biochemical nature.

It has been suggested that prior action by pectinmethylesterase (PME) which catalyses the hydrolysis of methoxylated galacturonosyl residues facilitates the activity of PG which has a preference for de-esterified pectin (33,35,38). PME has been found in mangoes (34), bananas (25), avocadoes (5), peaches (49), pears (37) and tomatoes (10). However, there is no clear correlation between its activity and cell-wall softening. Hamson (18) reported PME activity to be higher in unripe tomatoes whilst Hobson (22) observed 40% greater activity at the ripe stage.

Galactanase and arabanase

If the side chains of pectin which are thought to be galactans and/or arabinans and/or arabinogalactans are covalently attached to the cell wall as models indicate (28), then their endo-hydrolysis is necessary for solubilization of pectin in fruits devoid of PG. Such galactanase or arabanase activity has not been clearly demonstrated in fruits. Although unable to positively identify these enzymes in Ngowe mangoes, John (27) suggested their presence in a crude 3M LiCl extract from the walls of ripe fruits. This preparation which apparently did not contain PG was able to solubilize large polysaccharide molecules and a range of monosaccharides from cell walls of the unripe fruit. No oligosaccharides were detected. The changes brought about in degraded cell walls were similar to that which occurs during normal ripening.

Exoglycosidases

The involvement of exoglycosidases in cell wall breakdown has not been established. This class of enzymes (particularly β-galactosidase and α-arabinosidase) is of interest because they are present in a number of fruits and increase many-fold with ripening (2,8). They may be responsible for the removal of galactose and arabinose from cell walls, but this has not been demonstrated *in vivo*. Tomato (43) and apple (6) β-galactosidase are able to hydrolyse cell wall galactans. Otherwise, studies have mostly used artificial substrates such a β-nitrophenyl-β-galactopyranoside which may not accurately reflect true *in vivo* activity. Multiple forms of β-galactosidase have been identified in tomato (43) and mango (27). As exoglycosidases can only remove sugar residues from the end of large molecules, their action alone could not solubilize polysaccharides from the cell wall. Thus, they are unlikely to be of importance in cell-wall softening, unless in a regulatory role. Following initial cleavage by endoenzymes such as galactanase or arabanase, exo-hydrolysis of the exposed end groups becomes possible. Subsequent removal of these side chains may enhance the activity of PG or PME by reducing stearic hindrance.

CONTROL OF SOFTENING

Hobson (21) suggested that ripening should be considered as a number of key processes taking place simultaneously, each one having its own control mechanism which is loosely coordinated with those of other processes. Control of softening is dependent upon understanding the mechanism by which it is brought about and its interrelation with other pathways. Traditionally, in biochemistry, analogues of substrates and the final product of reactions have been used to inhibit enzymatic catalysis. Theoretically, it is possible to reduce softening by inhibiting key enzyme(s) involved in this process, particularly at the initiation stage. However, little attention has been paid to this possibility. It is likely that cell-wall degradation may have a regulatory role in other processes occurring inside the cell. Control may be effected by solubilization of attached enzymes which may then partake in the furtherance of ripening by acting on substrates present in the cytosol. In support of this, results obtained from studies on the action of PG using cell-wall substrates suggest that protein as well as carbohydrate material is released (25,50,51). Further, some reduction in firmness of fruits has been observed prior to incipient ripeness (5,7).

Modification of substrate is another strategy that could be employed to control softening. Calcium is mainly located in the cell wall of plants (18) and plays a crosslinking role in the structure of pectin (28). Addition of this ion could have rigidifying effect on cell walls and stearically obstruct enzymes such as PG from reaching their sites of action. Alternatively, direct inhibition of enzymes could be possible. For example, *in vitro* studies of tomato pectic enzymes show that maximal activity of PME had a requirement for Ca^{2+}, whereas PG activity was inhibited by concentrations as low as 10^{-7} M (57). Application of Ca^{2+} has been shown to inhibit normal ripening of several fruits (12,52,56), however at higher concentrations skin injury and secondary microbial growth results (53).

Once the enzymology of softening is better understood, control could be executed at the genetic level by inhibiting synthesis of key enzymes. This opens up the possibility of delaying the onset of ripening rather than slowing down the ripening process once it has already started as present-day methods such as refrigeration do. Certainly, protein synthesis appears to occur at the climacteric stage during fruit development (13,15,29). This is supported by evidence of increases in the ratio of protein nitrogen to total nitrogen (24,48). It has not been established whether *de novo* synthesis of protein catalyzes the climacteric rise. However, reports of ripening-related changes in the levels of different transfer RNAs (36) and messenger RNAs (44,45) in tomato fruit support this contention. As previously mentioned, PG is synthesized *de novo* in tomato shortly after the onset of ethylene production.

Traditional methods for controlling ripening and thus softening such as low temperatures and modified atmospheres have been tried and tested over the years. Although these can be refined to give increases in storage life of fruits, they are unlikely to provide any dramatic advances in postharvest technology. This requires the development of a base of scientific knowledge which offers more than is currently known today. There is therefore a need to invest in long term projects aimed at understanding ripening at the molecular level to provide information for future exploitation. At the same time, research directed at fine tuning present-day technology to fulfil more immediate requirements also needs to be carried out.

REFERENCES

1. AHMED, A.E., LABAVITCH, J.M. 1980. Cell wall metabolism in ripening fruit. I. Cell wall changes in ripening "Bartlett" pears. Plant Physiol. 65:1009-1013.

2. AHMED, A.E., LABAVITCH, J.M. 1980. Cell wall metabolism in ripening fruit II. Changes in carbohydrate degrading enzymes in ripening "Bartlett" pears. Plant Physiol. 65:1014-1016.

3. ALBERSHEIM, H. 1978. Concerning the structure and biosynthesis of primary cell walls of plants. Int. Rev. Biochem. 16:127-150.

4. ARIE, R.B., KISLEV, N., FRENKEL, C. 1979. Ultrastructural changes in the cell walls of ripening apple and pear fruit. Plant Physiol. 64:197-202.

5. AWAD, M., YOUNG. R.E. 1979. Postharvest variation in cellulase, polygalacturonase and pectinmethylesterase in avocado (Persea americana Mill, cv Fuerte) fruits in relation to respiration and ethylene production. Plant Physiol. 64:306-308.

6. BARTLEY, I.M. 1974. β -Galactosidase acticity in ripening apples. Phytochemistry 13:2107-2111.

7. BESFORD, R.T., HOBSON, G.E. 1972. Pectic enzymes associated with the softening of tomato fruit. Phytochemistry 11:2201-2205.

8. BRINSON, K. 1984. Changes in carbohydrates and glycosidases in mango fruits during ripening. PhD thesis, University of London.

9. CROOKES, P.R., GRIERSON, D. 1983. Ultrastructure of tomato fruit ripening and the role of polygalacturonase isoenzymes in cell-wall degradation. Plant Physiol. 72:1088-1093.

10. DENNISON, R.A., HALL, C.B., NETTLES, V.F. 1954. Pectin esterase activity of tomato fruits at different stages of maturity. Proc. Ann. Meet. Am. Soc. Horti. Sci., Florida, 51, 17.

11. DENNISON, R.A., AHMED, E.M. 1967. Irradiation effects on the ripening of Kent mangoes. J. Food Sci. 32:702-705.

12. FAUST, M., SHEAR, C.B. 1972. The effect of calcium on respiration of apples. J. Amer. Soc. Hort. Sci. 97:437-439.

13. FRENKEL, L., KLEIN, I., DILLEY, D.R. 1968. Protein synthesis in relation to ripening of pome fruits. Plant Physiol. 43:1146-1153.

14. FRY S.C. (1986) Ann. Review Plant Physiol 37 165-186 Cross-linking of matrix polymers in the growing cell walls of angiosperms.

15. GRIERSON, D., TUCKER, G., ROBERTSON, N. 1980. The regulation of gene expression during the ripening of tomato fruits. In "Quality in Stored and Processed Vegetables and Fruits" (P.W. Goodenough and R.K. Atkin, eds). pp, 179-191. Academic Press, New York.

16. GROSS, K.C., WALLNER, S.J. 1979. Degradation of cell wall polysaccharides during tomato fruit ripening. Plant Physiol 63:117-120.

17. GROSS, K.C., SAMS, C.E. 1984. Changes in cell wall neutral sugar composition during fruit ripening: a species survey. Phytochemistry 23:2457-2461.

18. HAMSON, A.R., 1952. Factors which condition firmness in tomatoes. Food Res. 17:370-379.

19. HASEGAWA, S., MAIER, V.P., KASZYCKI, H.P., CRAWFORD, J.K. 1969. Polygalacturonase content of dates and its relation to maturity and softness. J. Food Sci. 34:527-529.

20 HOBSON, G.E. 1964. Polygalacturonase in normal and abnormal tomato fruit. Biochem. J. 92:324-332.

21. HOBSON, G.E. 1979. What factors are involved in the onset of ripening in climacteric fruit? Comment. Plant Sci. 2:225-236.

22. HOBSON, G.E. 1963. Pectinesterase in normal and abnormal tomato fruit. Biochem. J. 86:358-365.

23. Comprehenisve review of cell wall changes/enzymes during fruit ripening: Huber D.J. (1983) Hort Reviews 5 169-219. The role of cell wall hydrolaser in fruit softening.

24. HULME, A.C. 1954. Studies in the nitrogen metabolism of apple fruits. The climacteric rise in respiration in relation to changes in the equilibrium between protein synthesis and breakdown. J. Exp. Bot. 5:159-172.

25. HUTLIN, H.O., LEVINE, A.S. 1965. Pectin methyl esterase in the ripening banana. J. Food Sci. 30:917-921.

26. ISHII, S., 1978. Analysis of the components released from potato tuber tissues during maceration by pectolytic enzymes. Plant Physiol. 62:586-589.

27. JOHN, M.A. 1984. Postharvest changes in the cell wall of mango fruit. PhD thesis, University of London.

28. JOHN, M.A., DEY, P.M. 1986. Postharvest changes in fruit cell wall. In "Advances in Food Research". C.O. Chichester, E.M. Mrak, and B.S. Schweigest (eds). Academic Press, Inc.

29. JONES, J.D., HULME, A.C., WOOLFORTON, L.S.C. 1965. The respiration climacteric in apple fruits. New Phytol. 64:158-167.

30. KNEE, M. 1973. Polysaccharide changes in cell walls of ripening apples. Phytochemistry 12:1543-1545.

31. KNEE, M., SARGENT, J.A., OSBORNE, D.J. 1977. Cell wall metabolism in developing strawberry fruits. J. Exp. Bot. 28:377-396.

32. LAZAN, H., ALI, Z.M., LEE, K.W., VOON, J., CHAPLIN, G.R. (in press). The potential role of polygalacturonase in pectin degradation and softening of mango fruit. ASEAN Food J.

33. LUH, B.S., LEONARD, S.J., PHAFF, H.J. 1956. Hydrolysis of pectic materials and oliguronides by tomato polygalacturonase. Food Res. 21:448-455.

34. MATTOO, A.K., MODI, V.V. 1969. Ethylene and ripening of mangoes. Plant Physiol. 44:308-310.

35. McCREADY, R.M., McCOMBE, E.A., JANSEN, E.G. 1955. Action of tomato and avocado polygalacturonase. Food. Res. 20:186-191.

36. METTLER, I.J., ROMANI, R.I. 1976. Quantitative changes in tRNA during ethylene-induced ripening (aging) of tomato fruits. Phytochemistry 15:25-28.

37 NAGEL, C.W., PATTERSON, M.E. 1967. Pectic enzymes and development of the pear (Pyrus communis). J. Food Sci. 32:294-297.

38 PATEL, D.S., PHAFF, H.J. 1960. Properties of tomato polygalacturonase. Food Res. 25:47-55.

39. PRESSEY, R., HINTON, D.M., AVANTS, J.K. 1971. Development of polygalacturonase activity and solubilization of pectin in peaches during ripening. J. Food Sci. 36:1070-1073.

40. PRESSEY, R., AVANTS, J.K. 1973. Separation and characterization of endopolygalacturonase and exopolygalacturonase from peaches. Plant Physiol. 52:252-256.

41. PRESSEY, R., AVANTS, J.K. 1975. Cucumber polygalacturonase. J. Food Sci. 40:937-939.

42. PRESSEY, R., AVANTS, J.K. 1976. Pear polygalacturonase. Phytochemistry 15:1349-1351.

43. PRESSEY, R. 1983. β-Galactosidase in ripening tomatoes. Plant Physiol. 71:132-135.

44. RATTANAPANONE, N., GRIERSON, D., STEIN, M. 1977. Ribonucleic acid metabolism during the development and ripening of tomato fruits. Phytochemistry 16:629-633.

45. RATTANAPANONE, N., SPEIRS, J., GRIERSON. D., 1978. Evidence for changes in messenger RNA content related to tomato fruit ripening. Phytochemistry 17:1485-1486.

46. ROE, B., BRUEMMER, J.H. 1981. Changes in pectic substances and enzymes during ripening and storage of Keitt mango. J. Food Sci. 46:186-189.

47. ROUHANI, I., BASSIRI, A. 1976. Changes in the physical and chemical characteristics of shahani dates during development and maturity. J. Horti. Sci. 51:489-494.

48. ROWAN, K.S., PRATT, H.K., ROBERTSON, R.N. 1958. The relation of high-energy phosphate content, protein synthesis, and climacteric rise in the respiration of ripening avocado and tomato fruits. Aust. J. Biol. Sci 11:329-335.

49. SCHEWFELT, A.L. 1965. Changes and variation in the pectic constitution of ripening peaches as related to product firmness. J. Food Sci. 30:573-576.

50. STRAND, L.L., RECHTORIS, C., MUSSELL, H. 1976. Polygalacturonases release cell-wall-bound proteins. Plant Physiol. 58:722-725.

51. TIGCHELAAR, E.C., McGLASSON, W.B., BUESCHER, R.W. 1978. Genetic regulation of tomato fruit ripening. Hort Science 13:508-513.

52. TIGWA, P.O., YOUNG, R.E. 1974. The effect of calcium on the ripening of avocado (*Persea americana* Mill) fruits. J. Amer. Soc. Hort Science 99:540-542.

53. TIRMAZI, S.I.H., WILLS, R.B.H., 1981. Retardation of ripening mangoes by post harvest application of calcium. Tropical Agric. 58:137-141.

54. TUCKER, G.A., GRIERSON, D. 1982. Synthesis of polygalacturonase during tomato fruit ripening. Planta 115:64-67.

55. WALLNER, S.J., BLOOM, H.L. 1977. Characteristics of tomato cell wall degradation *in vitro*. Implications for the study of fruit-softening enzymes. Plant Physiol. 60:207-210.

56. WILLS, R.B.H., TIRMAZI, S.I.H., SCOTT, K.J. 1977. Use of calcium to delay ripening of tomatoes. Hort Science 12:551-552.

57. WILLS, R.B.H., RIGNEY, C.J. 1979. Effect of calcium on activity of mitochondria and pectic enzymes isolated from tomato fruits. J. Food Biochem. 3:103-110.